BULLETIN

OF

THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

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SOME RECENT ADVANCES IN OUR KNOWLEDGE OF THE DUCTLESS GLANDS

[FIRST LECTURE ON THE KOBER FOUNDATION[1]]

JOHN J. ABEL

Professor of Pharmacology, The Johns Hopkins University

Whoever departs in a striking way from the normal human being, whether it be in respect to stature, color, gait or disproportion between limbs and trunk, or whoever exhibits a facial oddity, as bulging eyeballs or massive lower jaw, at once arrests the attention. A beard on a woman, a swollen neck, extreme height or a dwarfish stature will be noted by every passer-by. The tales that fascinate our childhood deal with fabled giants, one-eyed perchance, like Polyphemus of old, and capable of monstrous feats of strength, or of dwarfs and gnomes, "the little people." And our youngsters, when at the circus, seek out the dwarf and giant, the bearded lady, the 600-pound man and the living skeleton.

[1] Delivered upon Appointment by the Association of American Physicians at Georgetown University, Washington, D. C., March 28, 1925. At the thirty-ninth annual meeting of the Association of American Physicians held at Atlantic City, May 6, 1924, announcement was made of the establishment by Professor George M. Kober, Dean of the Medical Faculty, Georgetown University, Washington, D. C., of a Lectureship and Medal. The communication sent by Dr. Kober reads in part: "In commemoration of the fiftieth anniversary of my graduation in medicine, on March 8, 1923, I created an Endowment Fund to be held in trust by my *Alma Mater*, Georgetown University, Washington, D. C. The interest of this fund is to promote in a modest way research work among the members of the various associations with which I have been associated for many years." The Foundation provides for "a gold medal to be awarded annually to a member of the Association of American Physicians" and for annual awards for lectures by men who have contributed to the progress and achievement of the medical sciences or preventive medicine. The lectures to be delivered under the auspices of Georgetown University in Washington, D. C., and to be published as a continuation of a series of lectures inaugurated March 28, 1920, as a vindication of animal experimentation." Further details concerning the Foundation are given in the Transactions of the Association of American Physicians, 1924, vol. xxxix, page xxvii.

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These extreme departures from the average are numerically few, but those of lesser degree are visible on every hand to the trained eye, and the two classes together have assumed great importance in medicine as well as in general biology because they find their explanation in the over- or under-activity of certain small structures located at various points in our bodies and about which very little was known until the last few decades—the so-called organs of internal secretion, the study of which is now occupying the attention of scientists everywhere.

I hope to make it quite clear to you as I proceed that, in the words of Barker, "We are simultaneously, in a sense, the beneficiaries and the victims of the chemical correlations of our endocrine organs." These organs produce definite and specifically acting, indispensable chemical products, which are poured into the blood and which modify the development and growth of other unrelated organs, as well as of each other, more especially during embryonic and early life, and which generally greatly affect the entire metabolism, that of the nervous system included. These products of internal secretion are now generally called hormones, in accordance with the suggestion of Professor Starling.[2]

THE MOST IMPORTANT ORGANS OF INTERNAL SECRETION

The organs and structures producing an internal secretion are very numerous, and their number is constantly being added to. I shall not here give the entire list, but shall enumerate only the most important of them, which are as follows:

Thyroid—a small trilobed organ set astride the windpipe below the larynx, weighing in man 35 grams or $1\frac{1}{4}$ ounces. It is quite impossible to characterize the functions of this important organ in a single sentence. It has been definitely established, however, that an in-

[2] At this point lantern slides were shown of examples of thyroid disease in human beings and in animals, and statistics were given showing the great economic losses that result from the prevalence of such abnormalities in the so-called "thyroid belts" of different countries. Lantern slides were also shown of experimentally induced cachexia thyreo-priva; of diseases caused by abnormalities of the hypophysis cerebri, as acromegaly, gigantism and dystrophia adiposo-genitalis, of eunuchoid states and of striking alterations in the secondary sexual characters consequent upon pathological states in certain endocrine organs.

creased rate of metabolism is associated with an over-function and a decreased rate of metabolism with an under-function of this gland.

Parathyroid glands—very small organs, each about the size of a hempseed, to use the descriptive designation employed in 1880 by their discoverer, the Danish anatomist Sandström. Of these organs we usually have four, two on each side in the neck, closely adherent to the dorsal surface of each lateral lobe of the thyroid gland. Like most of the other organs of internal secretion, these glands have an unusually good blood supply. Together they weigh about half a gram or 7 to 8 grains. The chief function of these minute glands is to control in some as yet undetermined manner the chemical combinations in the tissues of the calcium ion.

Pituitary body. The pituitary body, or hypophysis cerebri, is a structure about the size of a small cherry and weighs approximately 0.612 gram or 10 grains in the human being. It is made up of two main portions which differ in function and in embryological origin. It is attached to the base of the brain by a stalk, and rests in a hollow in the sphenoid bone—the sella turcica. Its functions will be described in a later section.

The two suprarenal bodies—or adrenal glands, little yellowish structures, shaped somewhat like a cocked hat, each fitting snugly on top of its corresponding kidney. The two organs together, in a fully developed man, weigh less than one-third of an ounce. They, like the hypophysis, are double structures; each consists of a medullary or inner portion and a cortical or outer portion, which are, genetically speaking, two entirely different organs. These small structures are among the most vascularized organs in the body, as there passes through them six times their weight of blood in a minute. Their functional significance will be discussed later.

The pancreas—weighing in the human being about 80 grams or approximately 3 ounces, has not only an internal secretion, insulin, which is given up by certain cells directly into the blood-stream, but also an external secretion which is poured into the lumen of the upper intestine and which contains very important digestive ferments. The internal secretion, the now well known insulin, is derived from groups of cells named the islets of Langerhans.

The sex glands—which, in human beings as also in higher animals,

have a dual function—the formation of the reproductive-germinal cells (spermatozoa and ova) of their external secretion, and the elaboration of principles of internal secretion, which are determining factors in the differentiation of the sexes.

The upper part of the duodenum—the mucosa of which produces the precursor of the hormone of Starling and Bayliss, known as secretin. It is the function of this hormone, which is produced by the action of acid on the precursor, to stir up the pancreas to pour into the intestine a copious supply of its special digestive ferments.

The liver—which is not always included among the organs of internal secretion but is deservedly so classed, if for no other reason than that the carbohydrates of our food are there stored as glycogen and held in readiness to be again mobilized and converted by a ferment back again to blood sugar to be used in the body as needed. In passing, let it be stated that the entire musculature of the body also constitutes a store-house for glycogen about equal in amount to that in the liver and, as has recently been shown, the manner of whose deposition and mobilization is analogous to that of the glycogen in the liver. The work of recent years with insulin, thyroxin and epinephrine proves to what a large extent the glycogenic function of this organ is under hormonic control. I leave out of consideration here the rôle of the liver in the production of other substances, such as those concerned in the clotting of blood. The external secretion of this all-important and largest among the glandular structures of the body is, of course, that complex fluid, the bile.

The thymus—an organ which, according to some investigators, has a doubtful position as an organ of internal secretion in the case of human beings, undoubtedly belongs to this class when it comes to some of the lower animals, such as birds.

A number of other structures, such as the pineal body, the spleen, the prostate, the placenta, the mammary glands, should be named among those concerning which there is a great deal of controversy as to whether they are, in fact, hormone-producing organs. Evidence is, however, daily accumulating in favor of the endocrine function of many of these organs in the lower animals, if not in man, and it may be stated with confidence that the list of substances which may properly be classed as hormones will be greatly extended in the future.

SOME NEWER HORMONES

Modern research, as is well known to you, has revealed the astonishing fact that we require for the complete nourishment of the body, in addition to the energy-furnishing and mineral constituents of food, minute quantities of other substances, which are called vitamins. In addition to the minuteness of the amounts required, there are many analogies between the physiological properties of these vitamins and those of the hormones now under consideration. We may dismiss the vitamins here with a simple statement that they may be regarded as hormones of extraneous origin, the exact mechanism of whose action is now engaging the attention of many competent investigators in this and other countries.

And the list of substances that act in very dilute solution in the animal body is constantly being extended. It is well known to you that the so-called tissue culture, that is to say, the growing of isolated fragments of tissue, such as fibroblasts from a fragment from the embryonic heart of a chick, was initiated by the discoveries of Ross Harrison in 1906-07. In the intervening years, Carrel and his pupils, the Lewises, and others, have greatly extended this field of research. Carrel and Ebeling (1922), for example, have cultivated a fragment of embryonic heart tissue for more than ten years, and in this long period the general character of the culture and of the cells has not altered—the cells remaining typical embryonic fibroblasts. During this time Ebeling has carried the original fragment of tissue through 1860 generations of sub-cultures with 30,000 cultures. Such an achievement would be quite impossible without the presence in the culture medium of a certain amount of tissue juice obtained from crushed chick embryos. There are present in this embryonic tissue juice certain substances which the growing fibroblasts require and which are also present, but in much smaller amounts, in the tissues of the adult chicken. It was found that extracts of the organs of adult chickens cause at first an increase in the mass of the embryonic fibroblast cultures, but after a few generations the fibroblasts cease to multiply and ultimately die. Carrel has suggested the term "trephones" (from τρέφω—I feed) for the growth-promoting substances of embryonic tissue juice and of leucocytic extracts, and

concludes that these substances may be properly classed as a group of growth-stimulating hormones. New problems are set here for the biochemist, whose task it will be to determine the chemical relationship of these more recently discovered hormones to the ones that have been longer known.

If we consider only the minuteness of the amount of an organic substance that produces effects in the living organism, or even *in vitro*, we should have to include the causative agents of anaphylactic shock, innumerable catalysers, ferments and toxins. These substances, however, are never classed as hormones, and they are mentioned here only as examples of a multitude of substances occurring in plant and animal life which are active in infinitesimal amounts. In the plant kingdom, however, hormones proper of various kinds appear to play an equally important, if not occasionally a greater, rôle than in the animal kingdom, but into this phase of the subject I cannot enter here.

HORMONES VERY POTENT SUBSTANCES

It will be shown presently that a striking characteristic of these products of internal secretions is their potency; in other words, even in very minute quantity they produce effects. While in some cases the physiological effect of a given dose of the hormone, as in the case of the medullary suprarenal capsule principle, follows immediately upon its administration, in other instances the bodily response, as in the case of the thyroid principle and the internal secretion of the sex glands, is much slower. It often requires years for the full development of the results of their increased or diminished action or entire absence, as is well seen in gigantism, acromegaly, goiter and castration—all instances in which there is either an excessive or a diminished output, or none whatever, of the hormone concerned.

THE MODE OF ACTION OF A GIVEN HORMONE ON A GIVEN TISSUE OR ORGAN DEPENDS UPON THE CHEMICAL OR FUNCTIONAL STATE OF THE LATTER

In almost all cases of hormone action we have to consider not merely the hormone itself but also the receptivity or functional state

of the organ or organs which are to be affected. To give an example from clinical medicine in reference to this point: Keith suggests that the reason the hands and feet in acromegaly respond so easily and disproportionately to the growth hormone of the anterior lobe of the hypophysis lies in the fact that these organs are in more constant use and therefore respond more vigorously to this sensitizing growth hormone. This is only an exaggerated manifestation of the normal process, says Keith, and is due to flooding of the body with this hormone, for nothing is more certain than that hard manual labor will normally itself produce marked growth responses in the hands and feet, but this normal functional response never approximates to that seen in acromegaly.

Innumerable examples from the studies of modern pharmacologists and physiologists might be cited in support of our statement that the action of a given hormone is conditioned by the functional, or, if you like, the chemical state of the organ or tissue upon which it acts. An oft cited example was discovered by Dale some years ago. He found that, after the intravenous injection into a cat or dog of a medium dose of ergotoxin and after waiting for the induced vascular responses to subside, an entirely abnormal response now follows an injection of an ordinary dose of the adrenal medullary principle. In place of the usually observed vasoconstriction and the resulting rise in arterial pressure, the exactly contrary physiological response is observed, namely, a prompt and decided fall of arterial pressure and a lessening of the heart rate. Later investigations showed that this reversal in the action of the adrenal hormone following a harmless dose of ergotoxin is not confined to the cardio-vascular response to the hormone but is equally true of its other effects. The liver, for example, will now no longer pour out an increased amount of sugar into the blood as before the administration of ergotoxin. The work of recent years has shown that similar alterations in the activity of hormones in general are induced by any one of a number of organic or inorganic substances. The presence of one of these modifying agents in a given tissue may so alter its response to a given hormone that one may note either an increase or a decrease in its action, or, as in the case just cited, the exact opposite of its usual action.

The elusive circumstances that underlie these abnormal responses

are often overlooked or misinterpreted, and consequently two or more separate physiological principles are sometimes postulated in place of one, as it seems more rational to do this than to credit a single hormone with a multiplicity of powers, not to mention the paradoxical property of having both positive and negative qualities. Thus, Schafer and his collaborators early demonstrated that an aqueous extract of the posterior lobe of the hypophysis cerebri induces a diuresis upon intravenous injection. Later, other investigators, more particularly clinical authorities, discovered that such extracts also have a marked antidiuretic action, a physiological property of which frequent use is now made in medical practice in moderating the excessive urination of diabetes insipidus. It was natural at this time for investigators to attribute these opposite actions to two distinct principles, one a diuretic, the other an antidiuretic substance. After I had suceeded in isolating the hormone of the posterior lobe of the hypophysis in the form of a highly active (if still somewhat impure) tartrate, this view could no longer be maintained. Dr. Geiling and I were able to produce either diuresis or suppression of urine at will in our experiments on rabbits. A diuresis which could be induced repeatedly and as often as desired was readily obtainable with rabbits that had been previously fed for five days only with succulent vegetables, cabbage, spinach, etc., whereas animals fed without special attention to the nature of the food reacted to injections of the pituitary tartrate only with suppression of urine. The latter action is what might be called the normal action of the hormone, while its opposite or inverted action, of secondary importance and of short duration only, is observed when the chemical composition of the blood and tissues has undergone qualitative and quantitative alterations in consequence of a week's diet on watery vegetables. It is thus evident that one and the same hormone may act under given conditions in one way upon one animal and in a precisely opposite manner upon a second animal of the same species.

Other analogous conditions under which this essentially antidiuretic hormone may induce a temporary diuresis are found in the administration of highly diuretic agents, such as sodium chloride, urea, and glucose, as also in the employment of certain anaesthetics. In reference to the manner of action of this hormone under these and other

conditions the recent work of Fromherz, Molitor and Pick, Mackersie, Smith and McClosky, Stehle and Bourne, Craig, Starling and Verney, and others, should be consulted.

It has been shown repeatedly in recent years that the several hormones also mutually influence each other's action much in the manner just described. It may be stated in general terms, then, that a preceding or concomitant event, if I may use the term, conditions the action of a given hormone.

It has been known for a number of years that extracts of the posterior lobe of the hypophysis induce in rabbits, less certainly in dogs, a glycosuria of brief duration (Borchardt), and that in hypophyseal deficiency, whether occurring clinically or produced experimentally, a high tolerance for sugars is acquired, together with a tendency to the accumulation of fat (Crowe, Cushing and Homans). More recently, Burn has discovered that the simultaneous injection of a pituitary extract and insulin prevents in starved rabbits the fall in blood sugar content that inevitably follows the administration of the latter hormone, and states that the doses of pituitary extract used by him cannot of themselves produce a rise in the blood-sugar content sufficient to explain this inhibition of the action of insulin as being the result of an algebraic summation. Extracts of the anterior lobe of the hypophysis or of other tissues produce no such effect.

It was further found that pituitary extract removes the symptoms of hypoglycaemic convulsions and causes a rapid elevation of the blood sugar, and that the effect of a small dose of insulin is greatly increased by previous injection of ergotoxin. This investigator also furnishes confirmatory evidence of the following points that had previously been established by others: (*a*) pituitary extract inhibits epinephrine hyperglycaemia and glycosuria; (*b*) ergotoxin abolishes epinephrine hyperglycaemia and glycosuria: (*c*) insulin greatly reduces the hyperglycaemic action of epinephrine.

The relation between the two hormones, epinephrine and insulin, has recently been studied by Cannon and his collaborators by means of new methods. These investigators make use of the denervated heart of the cat, that is to say, a heart that has had its connections with all the parasympathetic and sympathetic fibers severed, as an indicator for an increased output of epinephrine, since such a de-

nervated heart is subject only to influences via the blood stream. In cats thus prepared, insulin increases the rate of the heart beat. As the sugar percentage continues to fall, the heart rate continues to rise, the increased rate going as high as 36 beats per minute. The injection of glucose intravenously, when the heart rate is augmented and other evidences of sympathetic activity are present, is promptly followed by the disappearance of all these signs. After removal of one suprarenal gland and denervation of the other, or after removal of both glands, the injection of insulin caused no acceleration of the heart, although the usual hypoglycaemia occurred. This increased heart rate is explained as being due to a direct action of insulin on the adrenal medulla, in consequence of which an increased amount of epinephrine is poured into the blood stream. Confirmatory evidence of such a "sympathomimetic" action of insulin is seen by Cannon in the facts that mydriasis and erection of hairs on the tail occur in cats treated with insulin, and Yukujiro Abe, who has worked out this adrenaline-insulin action in Trendelenburg's laboratory, also concludes that the increased output of epinephrine is only one of the symptoms of a general stimulation of the sympathetic system and points out, as Cannon has done, that in human beings mydriasis, increased perspiration and pallor of the skin are among the symptoms noted after large doses of insulin.

It should be stated that G. N. Stewart makes the criticism that "it is very risky to assume that changes in the rate of the denervated heart occurring under such complex conditions, in the midst of such metabolic disturbances, are necessarily due to alterations in the rate of epinephrin output" (*Physiological Reviews*, iv, 1924).

The work of Cannon and his collaborators finds confirmation in the researches of Abe. This investigator measured the changes induced by insulin in the horizontal and vertical diameters of the rabbit's pupil after previous removal of the superior cervical ganglion on one side, thus rendering the iris on that side hypersensitive to epinephrine (either introduced from without or secreted into the blood in the usual manner). In addition, atropine was dropped into the conjunctival sac of both the hypersensitive and the normal eye in order to forestall or obviate all possibility of pupillary alterations due to reflex or central action. In every one out of seven experiments of this char-

acter, the injection of insulin induced a very decided widening of the pupil, amounting in most experiments to an increase of more than one millimeter in each of its diameters. This dilatation begins very soon after the injection of the insulin and reaches its maximum at about the time (one hour or more) when the hypoglycaemia becomes marked, and persists, apparently, according to Abe's tables, as long as the insulin is effective. That this mydriasis is a specific insulin effect is shown by the results of dextrose injections, following which the widely dilated pupil is contracted to its former size. In these striking observations of Cannon and of Abe we have further proof of the fact that our hormones influence or condition each other's action. It will not escape notice that in the case of insulin we are dealing with a hormone which not only plays a paramount rôle in the metabolic changes which the carbohydrates undergo in the animal organism but which also, either in a direct or in an indirect manner, reacts in a striking manner with the sympathetic nervous system.

Let me close this section of my lecture with one more example of the influence of one hormone upon another. It has been shown by a number of investigators that when a thyroid extract is injected or when the thyroid gland in the living animal is induced by electric stimulation to pour an excess of its peculiar hormone into the blood-stream, a subsequent injection of epinephrine will induce a very much greater and more prolonged rise of blood pressure than is seen under normal conditions. The instances given above must suffice to prove that the hormones of the endocrine organs influence each other's action either in the way of augmentation or inhibition when administered in a manner appropriate to the experiment in hand.

At bottom, all of these newer revelations in respect to the varying responses of the organism to hormones and related drugs (especially those acting upon the autonomic nervous system) are essentially similar to the facts discovered by physiologists of an older day. In the field of vasomotor and other reflexes, for example, we have numberless examples of how differences in degree and kind of action, varying from plus to minus, are correlated with the intensity of a given stimulus, the conditions of temperature, the degree of anaesthesia, the extent of regeneration of cut nerves or other conditions of the experiment. The ultimate analysis of the mysterious and elusive

phenomena that are encountered in the field of endocrinology will no doubt continue to fascinate and challenge the intellect of investigators for centuries to come.

HORMONES IN RELATION TO BIOLOGICAL PROBLEMS

It is only in consequence of the modern detailed study of the action of these products of internal secretion (which, as everybody now admits, are substances of a definite chemical character) that a striking correlation has been established between them and certain perfectly definite morphological changes occurring either in the embryonic or in the adult state. This conception has been of the widest application in the different branches of biology as well as in medicine. The illustration just given above of how *all* the tissues of the hand, the bones, blood vessels, muscles, connective tissue, etc., hypertrophy in unison under the influence of a sensitizing hormone derived from the anterior lobe of the hypophysis, is a case in point. We may say, indeed, that it is now well established that, given an adequate diet with the needed vitamins, the growth and development of perhaps all the structures of the body, interrelated as they are in fact, are dependent upon the internal secretions which we are here studying. It would appear that in the absence of the necessary hormone these structures cannot remain normal, but regress or become atrophic. In other words, the maintenance of the normal condition is dependent on a continuous, if only infinitesimal, supply of the hormone in question. No better example of a dependence of this nature could be given than that existing between the ovary and the uterus; removal of the former is inevitably followed by atrophy of the latter, a fact which is explainable on no other assumption than that a hormone secreted by the ovary is carried by the blood to the uterus, the tissues of which are in some way sensitized by it so that it grows to full size and maintains this throughout the period of sexual activity.

A few words may be said in regard to the extent to which the doctrine of hormones has been applied to biological problems of a wider scope. Biologists have applied this concept to problems of evolution, heredity and morphogenesis. Sir Arthur Keith says that "rightly conceived, Darwin's theory of Pan-genesis is very much of the same character as the modern theory of hormones." This in-

vestigator, as also Bolk and others, from a study of endocrine disturbances, as acromegaly, coupled with embryological and anthropological studies, have expressed the conviction that the differentiation of mankind into racial types is based on the varying interaction of the internal secretory glands. From this point of view, "the whole endocrine system is conceived of as an organic unit, as a sort of controlling superorganization in our somatic organism, an *imperium in imperio*" (Bolk).

If the glands of internal secretion are functionally associated with each other, as is now believed to be the case, and if their harmonious operation leads to that equilibrium of nervous and metabolic processes that we call health (proper nutrition being taken for granted), then we may, even in the present state of our knowledge, assert that this equilibrium is the result of the action and interaction of the *specific products* or *hormones* of the endocrine organs. These chemical products, which may be regarded as autogenetic drugs, are integrating agents of a new type in comparison with the nervous system. Older writers employed the expressive phrase "*consensus partium*" to indicate the interrelationship of the various organs of the body. Even close to our time, this *consensus* was supposed to be effected only through the intermediation of the nervous system, a view tersely expressed by Cuvier when he said: "Le système nerveux est, au fond, tout l'animal, les autres systèmes ne sont là que pour le servir."

In this connection we might also recall the relations of the endocrine organs to the sympathetic-parasympathetic nervous systems, the systems that exercise both a controlling and an inhibiting influence upon the entire cardiovascular, digestive, metabolic and glandular functions of the body. Further reference will presently be made to this subject, but no extensive treatment of this topic, which, like other phases of endocrinology, is still in its infancy, can be given here.

The influence of the endocrine organs on immunity from various infectious diseases, if such exists, is at present little understood. Marine in a comprehensive paper in *Medicine*, on the Etiology and Prevention of Simple Goiter, points out how frequently thyroid enlargements are seen in early pulmonary tuberculosis, acute fevers, such as pneumonia, scarlet fever and diphtheria and in acute syphilis, and suggests that the thyroid reaction in these cases is explicable

on the grounds that this gland plays a rôle in the defensive mechanism against infection and intoxication. In this connection let me cite the following passages from a recent weekly letter of the Paris correspondent of the Journal of the American Medical Association: "Goitrous persons seem to resist tuberculous infection (Cassan, Hamburger, Betz). Couland has demonstrated that animals, in which thyroid deficiency has been brought about by roentgen rays, are especially resistant to tuberculous infection. Animals that have not been treated, but which are the offspring of hypothyroidean parents, likewise show a decreased susceptibility to tuberculous infection. In the various regions of Switzerland, too, it is asserted that there is an inverse relation between the frequency of goiters and the prevalence of tuberculosis." These assertions touch subjects that are quite outside my own fields of investigation and I can not venture to give an opinion as to their merits.

INTERRELATION OF THE ENDOCRINE ORGANS

Another and one of the most fundamental phases of our subject, one aspect of which has already been referred to, is the physiological and structural interrelationships of the endocrine organs. It is a pleasure to state that almost every week brings new discoveries giving factual proof of such interrelationship based on anatomical experiments. It is in this field, then, that the recent advances in our knowledge of the ductless glands are most striking. It is more especially when removal of one of the endocrine organs takes place in the prepuberal stage that marked alterations occur in one or more, generally several, of the remaining glands of this class. Among the remote consequences of these alterations we may then have marked changes in the body conformation. Nowhere are the mutual relationships of the organs of internal secretion better demonstrated than in castration experiments. Thus, it has been shown that in a whole series of animals, as in the rat, guinea-pig, rabbit, in horned cattle and in man, marked changes are found in the hypophysis after castration. This organ now increases in volume and in weight, and shows characteristic histological changes. In addition, changes have been noted in the thyroid, thymus, the pineal and the suprarenal glands. In the castrated cock, Fichera declares that he has found the hypophysis to be twice as large as in the normal animal.

Seitz has recently published the results of experiments made with the intention of learning what influence, if any, is exerted on the young *in utero* by certain of the glands of internal secretion of the mother. He removed from male and female rabbits the whole or part of the thyroid and of the adrenal glands and then carefully examined the progeny. The young of the rabbits with ablated thyroids were found to have a distinct hypertrophy of the pituitary gland, quite as in the very different experiments cited above. There were noted not only an increase in the weight of the gland, as compared with normally produced young rabbits, but also microscopical changes of a definite character. In the young of rabbits deprived of their suprarenal glands there was found an enlargement of the thymus and hypertrophy of the follicles of the spleen. It will be noticed that Seitz has found that it is more especially the allied glands of internal secretion of the young, rather than the homologous organ itself, that have undergone alterations consequent upon removal of a certain gland in the parents. The effects were not always seen, but Seitz concludes that they were sufficiently frequent and sufficiently definite in character to leave no doubt as to their significance. Should these observations be confirmed, the important fact would be established that a deficiency of a given hormone in the maternal organism is met by striking alterations in certain hormone-producing organs of the foetus.

It has long been known that during pregnancy the anterior lobe of the hypophysis is hypertrophied and that pregnancy and lactation cause enlargement of the thyroid gland. Experimental studies of recent years have made it clear that the thyroid gland, the hypophysis, the adrenals and the pineal gland stand in functional relationship with the sex glands and are consequently in a position to exert an influence on the secondary sex characteristics. This relationship is mutual in character, for it is found that the development of the sexual glands themselves depends on the state of development of the other endocrine organs.

Smith, Walker and Graeser in a preliminary communication to the Society for Experimental Biology and Medicine report some interesting experiments on the production of the adiposogenital syndrome. They destroyed the pituitary gland in rats by injection of a 10 per cent

solution of chromic acid, and this resulted in the development of Fröhlich's syndrome. Subsequent removal for diagnosis of one ovary and the corresponding horn of the uterus showed no corpora and no follicles. Later treatment of this animal with anterior hypophyseal substance caused ovulation to occur, as was revealed by vaginal smears taken at different times. At autopsy this animal showed a normal-sized ovary with many corpora and large follicles.

The experiments of Smith and Allen on amphibian larvae have thrown light upon the interrelationship obtaining between the thyroid and the hypophysis, in that they have demonstrated that removal of the epithelial component of the hypophysis of the anuran tadpole invariably alters, in the course of some weeks, the structure of the thyroid "so that in place of the normal, robust, colloid-filled gland, as seen in a normal unoperated control, a strikingly atrophic thyroid is seen. The vesicles, containing a small amount, or no colloid, are few in number, small and lined by low epithelium. . . . . Developmental studies have shown that this atrophic appearance is due to a developmental fault rather than to actual regressive changes" (Smith and Smith). The two investigators just cited have found that the atrophic gland of hypophysectomized tadpoles can be restored to the normal condition by the intraperitoneal injection of moderate amounts of the fresh anterior lobe of the bovine hypophysis, while injections of the pars intermedia or neural lobe invoke no thyroid response in such tadpoles. Hypophysectomized tadpoles also metamorphose when injected with fresh anterior lobe substance, thus again indicating that the thyroid has been activated.

We may now take a recent illustration from clinical medicine which illustrates very clearly the influence of one upon the other glands of internal secretion. Holmes, in the *Quarterly Journal of Medicine*, reports the case of a young woman, aged 24, suffering from a slowly growing tumor of the cortex of the right suprarenal gland. This neoplasm, benign in character and made up practically of normal cortical tissue, produced very marked changes in the sexual organs (atrophy of the uterus, overgrowth of the clitoris); the sexual functions were deranged (menstruation ceased for nine years); the secondary sexual characters were strikingly altered (abnormal growth of hair, atrophy of the mammae, change in the distribution of fat, and mascu-

line appearance of the limbs), and psychical disturbances were manifested (loss of erotic feelings and lack of modesty). Prior to the onset of these symptoms and up to the age of 17, the patient was in excellent health, a handsome, well-built girl, with a rather fair complexion, delicate skin and a profusion of brown hair on her head. When she was 13 years old menstruation commenced and continued regular and normal until her seventeenth year, when it ceased abruptly. Her breasts were well developed at the age of 14 years. The abnormal and excessive growth of hair commenced at about 19 years of age. Her general health remained undisturbed, but from the time her menses ceased she had gradually lost weight and become flat chested. There also occurred a marked alteration in her mental as well as in her physical character. Before any physical change was observed she was very fond of male society and had engaged in the ordinary flirtations of her age and class. When her menses ceased, she lost all interest in the opposite sex and preferred the society of women. At the age of 24 the patient was first seen by the physician who records the case. The most striking feature about her was the excessive growth of hair on her chin, lips and cheeks, distributed about the same as in an adult male. The hair on the upper lip was straight, coarse and brown, while that on the chin and in the area of the male beard was more reddish in color and curly. The limbs, especially the lower, were also covered with short crisp dark hairs, such as are often seen in the male but never in the normal female. The pubic hair extended up the abdomen and was continuous with similar coarse curly hairs on the chest between the atrophied breasts. On the back there was a soft downy growth similar to that frequently seen in young male adults. Her general appearance and configuration strongly suggested masculinity, though there still remained evidences that she had been a handsome young woman. Her face was rather lean for a girl of her age, the lower jaw being unusually distinct and the malar bones rather prominent. Her hands were massive, her fingers thick and distinctly masculine, and the muscles of her arms stood out as they do in a thin but healthy young man. The masculine appearance was even more prominent in the lower limbs. especially in the buttocks and thighs, where the subcutaneous fat which gives the characteristic shape to the female hips

was more or less absent. The muscles of the thighs and buttocks stood out, when contracted, as they do in an athletic male. The lack of subcutaneous tissue on the trunk also produced a striking resemblance to a boy of poor physique. Her breasts were small and contained little or no glandular tissue. The clitoris, on the other hand, was very large and projected between the labia. The uterus was small. Her voice had not changed. The sella turcica was not enlarged. The thyroid gland seemed normal in size. Her glucose tolerance was slightly raised. She was intelligent, and while under observation never exhibited any emotional or intellectual disturbances. She exhibited less shyness and modesty when undressed and examined than might be expected from a girl of her social class. Various glandular extracts (in turn, thyroid, pituitary and ovarian) were used without producing any noticeable alterations in her condition. She next complained of occasional pain in the right side of her abdomen and examination revealed a swelling there which later was diagnosed as a tumor and was removed. The patient bore the operation well and recovered rapidly. The neoplasm was benign in character and was made up practically of normal cortical tissue. It is a justifiable assumption that in this case an excessive amount of the cortical secretion was constantly passing into the general circulation.

All the above symptoms, collectively termed *virilism*, disappeared in a relatively short period after the surgical removal of the tumor. The patient menstruated 36 days after the operation and has continued to do so regularly. The clitoris has returned to normal size. The abnormal hair began to fall out shortly after the operation and has entirely disappeared. The hair on her scalp fell out extensively three months after the operation, but grew again and was now even more luxuriant and of darker color than it was formerly. Her breasts have developed and her configuration has become feminine in character again. Nine years after the operation she is in every respect still a perfectly normal woman.

We have here clear proof that benign tumors of the suprarenal capsule, of a type leading to hypersecretion of the cortical hormone, can influence the development of the sexual system, induce excessive growth of hair, with male distribution, cause hypertrophy of the external genital organs and produce other symptoms of virilism.

Similar cases affecting the female can be found in the medical literature of most countries, and numerous examples of sexual precocity in very young males, with a premature development of all the secondary sexual characters, associated with cortical tumors are also recorded.

In the examples given above we have merely touched the fringe of this far-reaching question of the interrelationship of glands. In the literature of clinical medicine one finds described many instances of this kind, these cases being frequently referred to as cases of multiglandular syndrome. In general, according to Timme, "the whole aspect of multiglandular syndrome is more or less confusing at the present stage of our knowledge," and Lisser writes: "As a matter of fact, the majority of endocrinopathies which we are justified in diagnosing exhibit a predominantly uniglandular basis, which anyone properly trained in endocrine diagnosis should be able to recognize. A firm foundation for incretory therapy demands a sharply defined analysis of the symptoms and signs, with emphasis on the gland primarily responsible."

THE SUPRARENAL GLANDS

It will now be in place to consider certain physiological and chemical properties of a few of these endocrine organs. To take first the suprarenal glands. Seventy years ago a distinguished clinician of Guy's Hospital in London, Dr. Thomas Addison, observed in his clinic (over a term of years) a class of patients "with a very remarkable form of general anaemia" and with more pigmentation of the skin than is normal in northern Europe. He summarizes the condition as follows:

> The leading and characteristic features of the morbid state to which I would direct attention are anaemia, general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach and a peculiar change of colour in the skin occurring in connection with a diseased condition of the suprarenal capsules. . . . . This singular dingy or dark coloration usually increases with the advance of the disease; the anaemia, languor, failure of appetite and feebleness of the heart become aggravated; a darkish streak usually appears on the commissure of the lips, the body wastes . . . . . the pulse becomes smaller and weaker, and without any special complaint of pain or uneasiness the patient at length gradually sinks and expires.

The above brief description gives the cardinal symptoms of this "by no means very rare" but almost invariably fatal malady named, in honor of the discoverer, Addison's disease. After careful clinical and pathological investigations Addison stated that he could find no proof of organic changes anywhere in the body, except in the suprarenal glands. At autopsy serious lesions in these organs, sometimes carcinomatous, sometimes tuberculous in character, were always found.

Shortly after the announcement of Addison's discoveries (1856) a French physiologist, Vulpian, discovered that an extract made from the crushed suprarenal glands of animals and of human beings gave a striking emerald-green color on the addition of ferric chloride. No other tissue, either of the higher or lower animals, gives this reaction. These discoveries had no notable consequences until many years after, when in 1894–5 Oliver and Schafer and, independently of them, Cybulski and Szymonowicz, demonstrated that suprarenal extract contains a substance which in minute concentration causes a remarkable rise, of brief duration, in the blood pressure, in consequence mainly of a powerful constrictor effect on the arterioles of the body. Investigators soon showed that the substance responsible for this vaso-constrictor effect is found only in the medulla of the suprarenal gland, and not in its cortical portion. Subsequent researches showed that the substance causing the cardio-vascular changes is a chemical principle which easily oxidizes on exposure to the air, turning first pink, then brown and finally yielding a dark amorphous substance, a so-called melanin. It is this principle also which after oxidation is responsible for the dark color of the medullary portion of suprarenal glands which, after removal from the body, have lain exposed to the air for some time. The ancient anatomists referred to this softened and darkened medullary portion of the glands as *atra bilis*, or black bile. This substance, as we now know, is an amino-alcohol of a relatively simple constitution and goes by the name of dihydroxymethylaminoethylolbenzene,

$$(OH)_2C_6H_3CHOH \cdot CH_2NH \cdot CH_3$$

It is this one, among the many constituents of the medulla, that gives Vulpian's color reaction with ferric chloride. This substance, like

most of our powerful alkaloids, turns the plane of polarized light to the left. It is now made synthetically, and medicine is no longer dependent on the adrenals of the ox or sheep for its manufacture.

No other principle of the body has of recent years received such exhaustive study along chemical, physiological and pharmacological lines. It passes in the medical literature under various names, as epinephrine, adrenalin, suprarenin, adrenin, etc. The official designation adapted by the U. S. Pharmacopea is epinephrin, a name first employed by me in my earliest investigations on this subject and derived from ἐπί upon, and νεφρός the kidney.

It is now well established that this hormone is continuously being given off in almost infinitesimal amounts to the venous blood and thence distributed throughout the body in a very high dilution but one which suffices to give tone to, to relax slightly, or to inhibit the activity of the various tissues of the body. There is still much discussion, however, as to the exact significance of the hormone under normal conditions as well as in circumstances of emergency. It is argued that it is not in itself essential to the continuance of life, since extirpation of the medullary portion, or chromophil tissue, of the suprarenal gland, which, as we have seen, is the source of the hormone, is not followed by death. It must always be borne in mind, however, that it is quite impossible to extirpate every trace of medullary or chromophil tissue, since tissue of this character is found not only in the accessory suprarenals but also, to a small extent, in every one of the sympathetic ganglia located in the abdominal and thoracic cavities. Suppose, for example, that an investigator has removed both suprarenals and all of the accessible accessory suprarenals, he will still have left behind a quite indeterminate, though admittedly a small, amount of chromophil tissue. Now, it is a well established fact that glandular organs in general, and especially the glands of internal secretion, have a very large factor of safety, which is only another way of saying that a small remnant of the gland can "carry on" in emergencies in place of the whole. Under the circumstances I cannot believe that it has been proved with certainty that epinephrine is not essential to the continuance of life. But perhaps this is a question of academic importance only, since all of the more recent work of pharmacologists and physiologists leaves no

doubt as to the functional significance of the hormone, be it only in cases of emergency.

Leaving now the question as to how far epinephrine exerts any function in the animal organism, I purpose to give you a brief summary of the extraordinary physiological discoveries that have been made in respect to this product in recent years. Foremost there is a striking parallelism in the action of epinephrine and excitation of the sympathetic nervous system. In a word, epinephrine stimulates or inhibits the action of practically all structures innervated by the sympathetic system in a way analogous to electrical stimulation of the sympathetic nerves going to that part. To give examples, it accelerates the rate of the heart beat (a sympathetic reaction), entirely comparable to that which follows electric stimulation of the accelerator or sympathetic nerves to the heart. It relaxes the stomach and the intestines. On the contrary, it causes increased tonus of the pyloric, ileocoecal and internal anal sphincter, as does stimulation of the splanchnic nerve. It causes a thick, viscid saliva to be secreted, an increase in lacrymal secretion, an increase in bile secretion, a tremendous increase in the sweat secretion of some animals, as in the horse and sheep (but has no effect on this secretion in most animals), contraction of the erectores pilorum, contraction of both the gravid and non-gravid uterus in many animals, relaxation of the non-gravid uterus, as in the cat, the rat, the mouse and the guinea-pig; contraction of the trigone and relaxation of the fundus of the bladder; relaxation of the bronchi, especially if they previously have been put into a state of spasm, dilatation of the pupil of the eye and exophthalmus, conversion of the glycogen of the liver and muscles into blood sugar, polycythaemia and relaxation of the coronary vessels. All these reactions may be produced by electrical stimulation of the appropriate sympathetic nerve to the part in question.

The point of attack of epinephrine in all of the above instances is not the nerve end itself but an intermediate anatomical unit, something which appears to be neither pure nerve tissue, nor yet a pure muscle or gland cell, but an unidentified something called the receptive substance, a myoneural or adeno-neural junction, as the case may be. It must be evident that we have in epinephrine a substance which must be classed in respect to its action and dosage with

our most powerful alkaloids. It is truly speaking, as I have said above, an autogenetic drug. And we are not surprised to learn that it has both antagonistic and synergistic relations not only to others of our autogenetic drugs, or hormones, but to many alkaloids or other drugs as ergotoxin, apocodeine, curare, atropine, physostigmine, histamine and the like. As a drug this hormone is of considerable importance in practical medicine. Its extraordinary ability to cause constriction of small arteries and veins whenever the drug can be applied to them, its power to cause relaxation of spastically contracted bronchioles and its power to resuscitate an arrested heart are to be noted among the facts that account for its widespread use. Naturally, a drug of such extraordinary potency and such a variety of actions must always be used with care and only by those who are endowed with intelligence. We are fortunate indeed that our suprarenal glands do not seem able, even in times of emergency, to throw out into the general circulation any but lower concentrations of this hormone. Life would be incompatible with the sudden liberation into the blood stream at any one moment of a considerable amount of it, say, 15 or 20 mgm. The two suprarenals of an adult contain together at the most only nine milligrams of the principle. After acute infections and various exhausting diseases this amount is very much reduced, but on recovery regeneration of the substance seems to occur within a few days.

It has been established with certainty that the cortex (or interrenal gland) of the suprarenal is essential to the maintenance of life, as death always occurs in animals of widely different species within about thirty hours after its removal. The results following ablation of the cortex, alone, therefore completely justify the original contention of Addison that the suprarenal glands are of the greatest importance in the animal economy; that life, indeed, cannot long continue after their removal or after their destruction by disease.

The assumption is generally made that the cortex produces one or more indispensable principles, or hormones, by virtue of which it functions in the organism, and this assumption is, no doubt, the correct one. Considerable work has been done along chemical and biological lines with the purpose of solving the riddles presented by this tissue, but thus far little of a positive character has resulted. There are

many facts that point to a connection between the cortical secretion and the full development of the sex cells. Schafer concludes that "there is no clear evidence that any kind of active autacoid substance (hormone) is produced by the cortical cells, and it is probable that their function is associated with the building up of metabolic products which are to find employment in other parts of the organism."

HYPOPHYSIS CEREBRI

The designation, hypophysis cerebri, is to be preferred to the more ancient one, *glandula pituitaria cerebri*, usually referred to as the pituitary body. The qualifying adjective, *pituitaria*, of the earlier name connotes a secretory function and recalls an earlier theory concerning an external secretion of the gland, which is now no longer tenable. The word *pituita*, as used by Cicero and other writers of the classical period of Roman literature, means mucus, slime or phlegm, and our predecessors of an earlier day, from Galen to Vesalius, developed the theory that the brain voids mucus and excrementitious matter through the infundibular stalk into the body of the gland whence these filter down in some way into the nose and upper pharynx. In my Harvey Lecture of 1924, to which I may refer those who may be interested, I have descanted further on this definite theory of an external secretion from the hypophysis. It is quite apparent that the term hypophysis connotes no theory of secretion, as the word pituitary does, but merely that the gland grows out below the brain, that it is an "undergrowing" organ at the base of the brain.

In giving the list of endocrine organs, I stated that the hypophysis consists of two main parts, an anterior and a posterior lobe. Closer examination, however, reveals smaller divisions which have been named respectively pars intermedia, pars tuberalis and the stalk or infundibulum connecting the gland with the floor of the third ventricle. The anterior lobe has the cellular structure of a glandular organ. The pars intermedia and pars tuberalis also present many of the features of glandular tissue; the pars intermedia consists of a very narrow strip of tissue from which chains of cells pass into the posterior lobe; and it is thought by some investigators that colloidal or other elements derived from these cells account for the physiological properties of the posterior lobe (Herring, Cushing and associates, and

others). Again, the problem is stated somewhat differently by others, who, while attaching less importance to the chains of invading cells, nevertheless refer the posterior lobe hormones to the pars intermedia for their origin, believing that the hormones are concentrated in some manner in the posterior lobe but never produced there (Houssay, Vincent and others, and, lately (1922), Maurer and Lewis). The posterior lobe is made up largely of so-called neuroglia tissue and gives little anatomical evidence of being a secretory organ. It is also poorly supplied with blood vessels as compared with the anterior lobe which is highly vascularized.

The human gland is a small structure, about the size of the tip of the little finger and weighs only 0.6 gram.[3] In comparison it should be noted that the bovine hypophysis weighs about 2.4 grams, and that in both instances the neural or posterior lobe is decidedly smaller than the anterior lobe, constituting, in fact, only about 1/6 of the entire hypophysis. I have already given you illustrations of several diseases that are associated with an alteration of function in these glands—gigantism, acromegaly, or combinations of these two states and a condition more frequently seen in youth than in the adult stage, known as dystrophia adiposogenitalis. This last is not infrequently met with and is characterized by an overgrowth of fat, sometimes quite enormous, by underdevelopment of the sexual organs, by nervous and mental manifestations, and various other mal-conditions. This dystrophy, as well as gigantism and acromegaly, is ascribed to an under-function of the pars anterior of the gland.

Clinical authorities and pathologists often encounter difficulties when they attempt to correlate what they call "the pituitary states," or diseases referred to above, with pathological lesions of the gland. Some authorities incline to the view that in the higher animals and in man we cannot always segregate such lesions from those of the nervous tissue constituting the floor of the third ventricle. Here at the base of the brain, in the hypothalamus, are important nerve centers that may influence blood pressure, regulation of the body temperature, carbohydrate and water metabolism. A few physiologists, as Camus and Roussy, have even maintained that the hypophysis itself is

[3] Two models, exact reproductions of the human hypophysis, and similar models of the bovine hypophysis were exhibited at this point to illustrate the text.

without significance in the production of the diseases referred to, and that these must be referred to pathological lesions of the floor of the third ventricle for their origin. I feel myself disinclined to accept this view. I cannot discuss this matter at great length here, but would refer those interested to my Harvey Lecture in which I have treated it more fully.

Let me only remark that experimenters have shown that in lower animals, as in the amphibia, the evidence in regard to the function of both the anterior and the posterior lobes of the gland is perfectly clear and conclusive. Remove the anterior lobe in the tadpole and there follows retardation of growth and failure to metamorphose. Feed the stunted tadpole an extract of the anterior lobe and growth begins again and metamorphosis occurs. We have here a fine example of replacement therapy which at the same time proves the existence of a function. So too in the amphibia the posterior lobe can be shown to have a function, because if we remove the entire gland, the animal loses control over the pigmentation of its skin. The operated frog no longer responds to light and shade, as does the normal frog, but remains pale. Administration to such a frog of an extract of the posterior lobe of other frogs, or of the ox for that matter, enables it again to regain control of its color under external stimulation. Removal of the anterior lobe alone is not followed by alterations in the pigmentary system.

Camus and Roussy and a few surgeons have no doubt had some examples of successful removal of the entire hypophysis in dogs which were not followed by any other symptoms than a temporary diabetes insipidus. It has been claimed that such operations prove conclusively that neither the anterior nor the posterior lobe exert any function whatever in the higher animals. I cannot believe that the position of these experimenters is quite sound. Dr. Geiling and I have lately observed that the floor of the third ventricle in the immediate vicinity and inclusive of the tuber cinereum contains a blood-pressure-raising and oxytocic substance quite indistinguishable from that present in the posterior lobe of the hypophysis. Since publishing these findings we have learned that Houssay had previously made observations of the same general character as to the extension upwards, into the floor of the third ventricle, of the posterior lobe principle

(see his monograph). In ablation of the gland by surgeons this accessory tissue cannot be removed. Left behind as it is in all operations on the hypophysis, it could still perform the function of the posterior lobe, since all endocrine organs possess a large factor of safety, that is to say, if only a little is left behind, this ordinarily suffices for the body's needs. Similarly, too, the pars tuberalis and the cells of the anterior hypothalamus always remain behind after a so-called total extirpation of the hypophysis in dogs. It has not been shown that these tissues cannot take over the growth-promoting function of the anterior lobe. In this connection we would also point out that there exists the pharyngeal hypophysis situated in the middle line near the junction of the nasal septum and the roof of the pharynx which is regarded by Lewis as an actively functioning gland, rather than as a rudimentary or involuting structure. It is also known to pathologists that cranio-pharyngeal duct tumors are frequently accompanied by Fröhlich's syndrome. Assuming that a pharyngeal hypophysis is present also in the dog, it will readily be seen that it might act as an accessory anterior lobe after the ablation of the hypophysis itself, as described above.

The pharyngeal hypophysis could thus supplant the pars tuberalis, which appears to be analogous in its structure to that of the anterior lobe. It is on such grounds that I cannot subscribe to the negative position of Camus and Roussy in regard to the function of hypophyseal tissue. I incline rather to the view that both lobes of the gland (including under these terms all cells, whether located in the hypothalamus or elsewhere, that produce specific pituitary principles) are functionally active in man and in the higher animals, as well as in the amphibia. On this assumption the anterior lobe would contain a chemical principle or principles which can influence the growth of our body structures, while the posterior lobe would also transmit to the blood a hormone capable of exercising various functions, if only in emergencies. One of these functions which is probably exercised continuously under normal conditions, but a diminished supply of which in the circulating blood leads to diabetes insipidus, manifestly has to do with the mechanism of water retention and transportation in the body.

CHEMICAL PRINCIPLES OF THE HYPOPHYSIS

The hormone of the anterior lobe has not yet been isolated as a chemical individual but the name "tethelin" has been given by Robertson to this suppositional but nevertheless real and existent principle. It has been repeatedly demonstrated that the mixture of substances which are present in an aqueous extract of this lobe produces striking effects in the metabolism and growth of hypophysectomized animals standing as high, in the biological series, as the rat. One can not doubt, therefore, that we have to do with a specific, but as yet unidentified, growth-promoting substance.

Greater progress has been made in the isolation of the specific principle of the posterior lobe. It must be stated that it is still a matter of controversy whether this lobe contains only one specific principle, endowed with several physiological properties, or whether there are three or more specific principles. My collaborators and I, on the basis of intensive experimentation extending over a series of years, have been forced to adopt the first alternative, namely, that there exists in the posterior lobe only a single important specific principle. Inherent in this principle are a surprising number of various physiological properties. Thus a tartrate of this principle, which we have isolated and for which we cannot as yet claim entire chemical purity, exhibits all of the diverse physiological activities of a well-prepared aqueous extract. Acting upon the entire cardiovascular apparatus constricting the arterioles and capillaries, it causes the well known significant and prolonged rise of blood pressure, exactly as does the aqueous extract. It acts in a remarkable way on the respiration when the dose is more than a mere minimum, causing the periodic respiration, known as Cheyne-Stokes respiration. Like the extract also, it acts on the kidney in two ways. In rabbits, for example, which have been fed for a week on cabbages or other succulent vegetables, it causes after each injection of a minute amount only a sharp but temporary flow of urine. Under the more usual conditions of laboratory experimentation on both rabbits and dogs, its more constant action is to cause a diminution or even a temporary cessation of urinary flow. This latter antidiuretic action is seen at its best in human beings in the disease known as diabetes

insipidus, and it is for the control of the excessive and annoying increase in urination and consequent increase in thirst that posterior lobe extracts find one of their most beneficial uses. Numerous trials in hospitals have now shown that when our tartrate is administered subcutaneously or in the form of a nasal spray to patients suffering from this form of diabetes relief is secured as promptly as when a well prepared aqueous extract of the posterior lobe is injected. Our tartrate also, like a good extract of the posterior lobe, darkens the skin of tadpoles that have been changed into pale tadpoles by removal of the posterior lobe of the hypophysis. Our isolated tartrate is, lastly, an extraordinarily powerful stimulant for all smooth muscles, as is well illustrated by its power to induce the virgin guinea-pig's uterus to contract. Here we may compare its action with that of the well-known amine, β-imidoazolylethylamine, more generally known as histamine, which up to the present time has been the most powerful oxytocic or uterus-contracting agent known to medical science. The best and final tartrates are found to be from 1000 to 1200 times more powerful in their action than the acid phosphate of histamine.

The chemical methods which we have followed need not be given here, as they are accessible in earlier publications from my laboratory, but there are a few other points which I might lay before you as bearing on the controversy of one versus several specific principles in the posterior lobe. In the first place, is it not very striking that our tartrate should have remained a multivalent principle after having been put through a long series of widely varying chemical operations? All of the physiological properties of our tartrate increase in intensity and in the same ratio, *pari passu* with the degree of purification. Is it likely that this would be the case, unless these properties are characteristics of one and the same principle? Exposure of the tartrate to normal sodium hydroxide for one hour at room temperature, completely destroys every one of its characteristic physiological actions. It is extremely unlikely that so comparatively mild a treatment as this should simultaneously destroy three or four separate principles. Boiling a solution of the tartrate with 1 per cent hydrochloric acid under a reflux condenser for half an hour is also destructive of all of these physiological properties, with the exception of the

oxytocic action, which is not completely abolished by this short treatment with acid.

Our contention that we have but one specific hormone in the posterior lobe is receiving much support from other quarters. Smith and McClosky conclude from their experiments that "the identical diffusion rate of the oxytocic, pressor and renal activities present in infundibular extracts argues in favor of their chemical identity, and confirms the view held by Abel and his collaborators on this matter." In another carefully conducted piece of research these investigators studied the effect of keeping a posterior extract for various periods of time at a temperature of 60°C. on both the pressor and oxytocic properties. One extract which was kept in the incubator for 1½ months at this temperature lost 80 per cent of its oxytocic activity, and as nearly as could be determined, the same loss was found in its pressor activity. Another extract kept under the same conditions for 3 months retained only a trace of its oxytocic property, namely 6 per cent, and likewise only an indeterminable trace of its pressor activity. Is it likely that two separate constituents should show, in equal degree such extraordinary sensitiveness to so mild a treatment? In agreement with what has been said above, is it not more likely that the two properties studied in these experiments, the oxytocic and pressor, are characteristics of one and the same substance, rather than that they represent two separate principles?

A recent communication of Leyko and Sikorski also gives strong support to our contention. These investigators compared the pressor and antidiuretic actions of the posterior lobe extracts on dogs with the oxytocic action of the extracts on the guinea-pig's uterus and found that "thes three actions go together." They conclude, "An extract giving a strong pressor effect acts with about equal strength upon the diuresis and vice versa. This seems to speak in favor of Abel's view that the extract contains only one main active principle acting in all three directions, and against the presence of a number of different active bodies in the gland, each acting only in one way." We see, therefore, that a great variety of experimental facts, chemical as well as physiological in character, lend support to my belief that there exists in the posterior lobe but one specific hormone, possessed, however, of manifold physiological properties. In opposition to this

view stands the work of Dale and Dudley, Dreyer and Clark, and Fenn. Dale and Dudley have in past papers maintained that there are three or even four specific principles. What has been said above may be considered as my reply to their contention, which rests mainly on the differential behavior of the assumed several substances toward solvents. Fenn, in a preliminary communication from Dale's laboratory, has recently announced that he can separate the oxytocic from the pressor principle by extraction of aqueous extracts of the gland with n-butyl alcohol. In an earlier paper with Rouiller I have described my experiences with this solvent in connection with the pituitary tartrate, and I would only state here that dry butyl alcohol has only a very slight solvent action for the tartrate and that the fraction of it which is soluble in the alcohol (after repeated exposure to it) has exactly the same physiological action as the part which remains undissolved. On the other hand, a somewhat watery solution of the alcohol dissolves the tartrate completely, failing here also to effect a separation of substances possessed of different physiological properties. Until I can have access to Fenn's detailed paper, I can only state that we have here for the moment what is apparently an irreconcilable difference.

I have given you above a rather concise but incomplete account of the structure, functions and chemical principles of the suprarenal glands and the hypophysis cerebri, but my description, such as it is, must make it evident to you what difficulties and intricacies are encountered in the study of any one of the organs of internal secretion. Progress even though it be slow is, however, being made in every department in this field. Never before were there so many investigators, chemists, biologists, pathologists, and clinicians engaged in one phase or another of this enormous subject. Already the usual output of special articles in this field numbers more than 3000 per annum. Undoubtedly, we may feel confident that the future has in store for us many striking revelations in this all inclusive field of the organs of internal secretion and their specific hormones.

Were there time I should attempt to give you a brief account of the recent investigations with respect to the thyroid, parathyroid, ovarian and pancreatic hormones. You are all more or less familiar with the fundamental chemical discoveries of Kendall in regard to the iodine-

containing hormone of the thyroid gland, and it may truly be said that, next to the medullary principle of the suprarenal gland, the thyroid hormone is best understood from the chemical as well as the physiological point of view. Until, however, its synthesis has really been effected, the investigator must remain somewhat doubtful as to its ultimate structure. In respect to the chemical nature of insulin, investigators have hitherto had nothing of importance to offer. There is every prospect, however, that this, the newest of the hormones, must soon yield the secrets of its composition and the rationale of its operations in the body.

The field of study from which I have selected a number of typical examples for your consideration this evening is replete with marvelous and mysterious occurrences, only a few of which are fully understood at the moment. The importance of the hormones, quite aside from their elusive nature, naturally induces to name them long before they have been isolated as chemical individuals. The name selected in a given instance generally connotes either an anatomical relation or a noteworthy physiological property of the hormone rather than some characteristic of a molecular type. With increasing knowledge many of the terms now in use, serviceable as they may be, as hormones, autacoids, hormazones, adrenalin, adrenin, epinephrin, thyroxin and insulin, will be replaced, though perhaps not entirely, by chemical terms. Thus, epinephrin, adrenalin, suprarenin, adrenin and other synonymous terms can mean only one thing, namely, that the designated substance is a special principle found only in the suprarenal gland. When chemists had finally solved the mystery of its molecular structure and had learned to prepare it synthetically, it was quite in order for them to name it 1, 2-dihydroxy-4^2-methylaminoethyl-4^1-olbenzene, $C_6H_3(OH)_2(CHOH \cdot CH_2NHCH_3)$—a designation which is short hand for much sound chemical science. There is much less opportunity for the profitable utilization of a given hormone on the part of quacks or deluded romanticists when once its chemical structure and its chief physiological properties have been elucidated than when its properties were still shrouded in mystery.

When once all of endocrinology shall have been worked up as carefully as has been the case with the medullary principle of the suprarenal glands (a consummation that will require many decades for its realization), the whole subject will be submerged in and become an integral part of chemical and biological science.

STUDIES ON THE PROTECTIVE SUBSTANCE IN ANTIPNEUMOCOCCUS SERUM

Study III. The Protective Substance in Types I, II and III Antipneumococcus Sera

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This study is a continuation of the subject reported in Studies I and II (1), (2) with special reference to a comparison of the protective substance present in Types I, II, and III antipneumococcus sera. In the previous reports it was shown that a large part of the protective substance in potent Type I serum is precipitated by dilution with water. The precipitate so obtained is sparingly soluble in water but very soluble in neutral salts. It would thus appear to have solubility characteristics similar to those of the classical serum euglobulin. In extending our work to Types II and III sera it was found that, with sera from some horses, results were obtained similar to those with Type I serum. However, many exceptions occurred. There were observed two apparent reasons for these irregularities: first, the Type II and III sera studied were of relatively low potency as compared with Type I serum; second, there occurred in the water-insoluble precipitate two substances, the one soluble in neutral salts and relatively high in protective value, the other insoluble in neutral salts and displaying action antagonistic to protection. Although complete analysis of the factors presented in this problem has not been made, certain aspects have been taken up and the results will be given in this and other studies in preparation. A review of the work by other investigators on the pneumococcus antibody is made in other publications (1), (2), (9), (11). It is sufficient to say that the investigations of Avery (3), Gay and Chickering (4), Chickering (5), and Huntoon (6) are important contributions on this subject, since they deal with endeavors to isolate and to study the protective substance.

In making the comparisons reported here on immune sera, we have attempted especially to maintain uniform conditions for all three

types. Only fresh serum without preservative was used. Protective titer in a series of dilutions was determined on duplicate mice, and was for the most part repeated four to eight days after the first test. An eight-hour broth culture of pneumococcus was used in all cases. With Type I the dose of organism was 0.05 cc. (0.5 cc. of 1–10 dilution) of culture of such virulence that 0.5 cc. of 1–10,000,000 dilution killed duplicate mice in 36 hours; with Types II and III 0.005 cc. (0.5 cc. of 1–100) of culture was considered a unit dose when the strain possessed the same virulence as Type I. All dilutions were made with a separate pipette for each member of the series.

The amount of protein in the various solutions and precipitates was estimated by their nitrogen content. The Gunning modification of Kjeldahl's method was used. In each test sufficient protein was taken so that at least 1 cc. of N/10 hydrochloric acid was required to neutralize the amount of ammonia released.

The following subjects were taken up, each one as a single experiment: (*a*) the amount of precipitate obtained at various hydrogen-ion concentrations from immune serum of the different types as compared with that of normal horse serum; (*b*) the effect of dilution with water on the yield of precipitate containing protective and agglutinating substances; (*c*) a fractionation of immune serum at various dilutions and hydrogen-ion concentrations; (*d*) a preliminary report on a toxic substance in immune serum which weakens the animal defense; (*e*) the effect on antibody of reprecipitation of a sodium chloride solution by dilution with water; and (*f*) the solubility of the water-insoluble precipitate at various hydrogen-ion concentrations from different types of immune sera, including a comparison with the solubility of euglobulin under the same conditions.

EXPERIMENT I. THE YIELD OF PRECIPITATE AT VARIOUS HYDROGEN-ION CONCENTRATIONS FROM IMMUNE AND NORMAL SERA

The fact that serum, when diluted with water, yields a precipitate is not a new observation, this having been observed by Panum (7) in 1852. Indeed unpublished observations of precipitation on dilution of immune sera, such as antipneumococcus or antimeningococcus serum, have also been made known personally to the writer by workers in this field. The interesting fact which has been reported in previous

papers (1), (2) is that this precipitate contains the major part of the protective substances present in the immune serum. The observation was also made in the course of prior work that, while the normal horse serum yielded a precipitate on dilution with water, the yield was by no means comparable in amount with that from the serum of animals strongly immunized against Types I, II, and III of pneumococci. The purpose of this experiment was to study further the differences in this respect between normal and immune sera.

One sample each of Types I, II, and III and of normal serum was tested in the following way: 100 cc. of serum were added to each of six diluents,

First, distilled water 400 cc.
Second, distilled water 380 cc. and N/20 hydrochloric acid 20 cc.
Third, distilled water 360 cc. and N/20 hydrochloric acid 40 cc.
Fourth, distilled water 340 cc. and N/20 hydrochloric acid 60 cc.
Fifth, distilled water 320 cc. and N/20 hydrochloric acid 80 cc.
Sixth, distilled water 300 cc. and N/20 hydrochloric acid 100 cc.

The mixtures were allowed to stand at room temperature until flocculation occurred, and then transferred to the ice-box overnight. The precipitate was then collected by centrifugalization and dissolved in 20 cc. of 3 per cent sodium chloride. All the precipitates were readily soluble, with the exception of the last two on the acid side. In these, both from immune and normal serum, a small amount remained insoluble. The series of dissolved precipitates was kept in the ice-box until tested for protective value and nitrogen content.

Chart I shows a pronounced difference in the nitrogen content of the precipitates from the same amount of the different sera. The results may be summarized as follows: Type I serum gives the greatest amount of precipitate, Type III next, then Type II, and Normal the least. With the exception of Type III, which failed to yield a precipitate at pH 5.2, these results are true throughout the entire series of hydrogen-ion concentrations. The hydrogen-ion concentration at which the greatest amount of precipitate is thrown down apparently varies with the different sera; with Type I it is pH 6.4 to pH 6.2; Type II, a broad zone from pH 6.8 to pH 5.5; Type III, pH 6.8 to pH 6.7; and Normal the same as with Type I, pH 6.4 to pH 6.2. The significant difference, however, between immune and

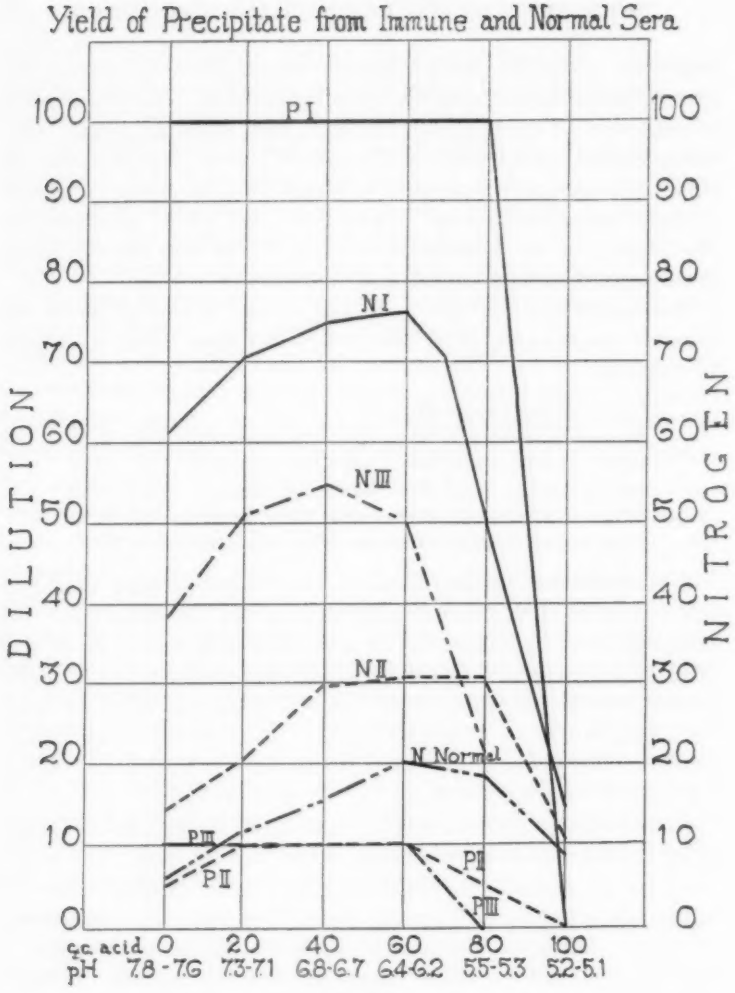

CHART I

PI, protection of supernatant of Type I; *PII*, protection of supernatant of Type II; *PIII*, protection of supernatant of Type III; *NI*, milligrams of nitrogen per cubic centimeter of dissolved precipitate Type I; *NII*, milligrams of nitrogen per cubic centimeter of dissolved precipitate Type II; *NIII*, milligrams of nitrogen per cubic centimeter of dissolved precipitate Type III; *N*, Normal—milligrams of nitrogen per cubic centimeter of dissolved precipitate, normal serum.

normal sera is the fact that the yield of precipitate from each immune serum was more abundant in the alkaline range of the series than the yield from normal serum (euglobulin). It is thus seen that there is not only an increased amount of water-insoluble protein in antipneumococcus sera, but that the zone of hydrogen-ion concentration in which precipitation is more complete is also broader in these than in the case of normal serum. Since the conditions of precipitation are the same, it may be inferred that we are dealing with either a modified euglobulin or pseudoglobulin or a protein of different characteristics from either of these. It should be borne in mind, however, that these results are based on only one sample of each serum. Comparisons with other experiments make it probable that with Types I and III these results are typical, and with Type II only true in case of weak immune sera. The precipitate from this specimen of normal serum is much greater than the average.

From Chart I it may also be seen that protective value does not parallel the amount of precipitate. This is especially observed with Type III serum, for at a hydrogen-ion concentration of pH 5.5 there is more protein, as judged by nitrogen, than in normal serum at the hydrogen-ion concentration of maximum precipitation (pH 6.4 to pH 6.2); and yet there is no demonstrable protective value. This is also true of Type I and II sera at a hydrogen-ion concentration of pH 5.2. Here again, although there is more protein in the precipitates than in normal, the protective substance is apparently absent. These observations further indicate that the protective substance, if it be associated with either pseudoglobulin or euglobulin, causes a change in their chemical properties.

EXPERIMENT II. THE RELATIONSHIP BETWEEN THE AMOUNT OF DILUTION WITH WATER AND THE YIELD OF PROTECTIVE SUBSTANCE

This experiment was undertaken for two reasons: first, because of observations that with many samples of Type II and Type III sera very little precipitate is obtained in the higher dilutions; and second, because of reference to the work of Izar and Caruso (8) in which they have reported that there was an optimal dilution of certain immune sera at which agglutinins, complement-fixation bodies, were pre-

cipitated. In performing this experiment a unit volume of serum was precipitated by water dilutions from 1–1 to 1–20, using an increment of one volume of water, in a series thus: 1–1, 1–2, 1–3, 1–4, etc., to 1–20. However, the results only from every other dilution gave significant differences. As seen from Table I, the nitrogen content in Type I shows that the same amount of precipitate was obtained by dilutions of 1–10 and of 1–20, whereas protective value and agglutination titer reached a maximum at a dilution of 1–18 to 1–20. Results with Type II serum are quite similar to those obtained with Type I with the exception of protection, in which there are two zones of high titer, namely, at dilution of 1–5 to 1–8 and again at dilution of 1–14 to

TABLE I

The relationship between the amount of dilution with water and the yield of protective substance

DILUTION	TYPE I			TYPE II			TYPE III		
	Milligrams nitrogen per cubic centimeter	Agglutination	Protective dose	Milligrams nitrogen per cubic centimeter	Agglutination	Protective dose	Milligrams nitrogen per cubic centimeter	Agglutination	Protective dose
1–2	0.257	1–30	1–40	0.284	1–2	1–2	0.259	1–2	0
1–4	0.401	1–45	1–80	0.718	1–3	1–20	0.487	1–6	1–5
1–6	0.436	1–80	1–100	0.998	1–5	1–20	0.497	1–7	1–5
1–8	0.457	1–100	1–125	0.998	1–10	1–20	0.490	1–7	1–5
1–10	0.471	1–120	1.125	1.005	1–10	1–20	0.504	1–7	1–5
1–12	0.436	1–110	1–150	1.11	1–10	1–20	0.490	1–7	1–10
1–14	0.443	1–110	1–200	1.02	1–10	1–40	0.518	1–7	1–10
1–16	0.453	1–120	1–200	0.977	1–10	1–40	0.469	1–7	1–5
1–18	0.451	1–150	1–200	0.914	1–10	1–40	0.455	1–8	0
1–20	0.453	1–150	1–200	0.793	1–12	1–20	0.371	1–6	0

1–18. The sera of this type, however, show irregularities: some of low protective value yielding no precipitate in the higher dilutions (1–14 to 1–18); others giving results comparable to those reported in this experiment, that is, a smaller yield of protective substance at dilutions of 1–8 to 1–12 than at dilutions 1–5 to 1–8. This Type III serum behaved more like Type I, for the maximal protection was found in the precipitate given at a dilution of 1–12 to 1–14. Other samples of Type III serum have been studied in which a dilution of 1–5 to 1–8 precipitated all the protective substances, as judged by the fact that

0.5 cc. of the supernatant lacked the power of protecting mice against a unit dose of culture.

It may be said in summary, that with Type I sera this experiment represents a general rule, while with Types II and III it exemplifies only a certain percentage of sera studied. Variations have been found with sera from different horses and also from various bleedings of the same horse. The explanation of these differences is not clear. Yet, as will be shown later, it may be in part due to toxic substances present in immune sera.

EXPERIMENT III. VARIOUS FRACTIONS OF WATER-INSOLUBLE PRECIPITATE

This experiment was undertaken for a two-fold purpose: to test the truth of the assumption that the water-insoluble substance precipitated from immune sera by dilution in water was different from the euglobulin of normal serum; and to ascertain the possibility of separating from the protective antibody the substance which has been found antagonistic to protection. That there is a substance in antipneumococcus sera which antagonizes the defense of the animal has been reported by Chickering (5), and has been observed many times in the course of this investigation. This has been seen in certain sera of Types I, II and III, especially from those horses which have been immunized only for a short time or in those which have been injected with large or excessive doses of antigen. In another publication (1) it was stated that not only did this substance appear in immune sera and in the precipitate obtained by dilution with water, but that it is also specific to type. Further work, a report of which will be given in a separate publication, has in the main confirmed this observation, the difference being a question of type specificity.

Inasmuch as the solubilities of the protective antibody and the precipitate containing the toxic material appear to be different from the solubility of euglobulin, it is probable that one procedure would serve the two aims of this experiment: namely, a fractionation of the serum by successive dilutions with water and with acid. For this experiment, sera of the different types were used as follows: Type I, a single bleeding 3500 cc.; Type III 3900 cc.; while with Type II there was a mixture of the sera from different horses aggregating 21 liters.

The following fractions from Type I serum were made successively: (1) by dilution 1–1 with distilled water; (2) by dilution 2–3 of the preceding supernatant, making a serum dilution 1–5; (3) by further dilution to make 1–10 and (4) by the addition of 30 cc. of N hydrochloric acid to 15 liters of the 1–10 supernatant. The precipitates were

TABLE II

Various fractions of water-insoluble precipitate (Type I)

(All mice received injections of 0.5 cc. of an 8-hour broth culture diluted 1–10)

FRACTION	VOLUME	MILLIGRAMS NITROGEN PER CUBIC CENTIMETER	NITROGEN IN PROTECTIVE DOSE	DILUTIONS OF SAMPLES							
				0.2*	1–10	1–20	1–40	1–80	1–160	1–320	1–640	1–1280
Dilution 1–1	37	19.32	0.06	18	S	S	S	S	S			
				18	S	S	S	S	S			
Dilution 1–5	198	21.17	0.008	18	84	S	S	S	S	S	S	S
				S	S	S	S	S	S	S	S	S
Dilution 1–10	11	13.97	0.01	18	84	S	S	S	S	S	S	36
				18	96	S	S	S	S	S	S	40
Dilution 1–10 with acid	43	7.54	0.046	84	84	108	108	108	60	60	36	
				96	74	S	S	S	60	60	84	
Serum control				S	S	S	S	S	S	20		
				S	S	S	S	S	S	60		
		1–10,000,000				1–1,000,000						
Control organism		60				84						
		84				84						

Numbers refer to hours of survival.

* 0.2 cc. injected of undiluted sample.

dissolved in 3 per cent sodium chloride in the volumes given in Table II. The protective dose of the various precipitates as well as of the control serum was twice estimated on duplicate mice. A study of the results from these titrations on the fractions obtained from Type I serum reveals certain facts: in the first place, inasmuch as the mice

injected with the high concentrations of the precipitates along with a unit dose of organisms died while those with lower concentrations lived, a substance antagonistic to defense is seen to exist in the first three fractions and possibly the fourth. However, in the second dilution 1–5, this substance per milligram of nitrogen is less than in the

TABLE III

Various fractions of water-insoluble precipitate (Type II)

(All mice received injections of 0.5 cc. of an 8-hour broth culture diluted 1–100)

FRACTION	VOLUME	MILLIGRAMS NITROGEN PER CUBIC CENTIMETER	NITROGEN IN PROTECTIVE DOSE	DILUTIONS OF SAMPLES					
				0.2*	1–10	1–20	1–40	1–80	1–160
Dilution 1–6	1200	7.056	0.044	18	36	S	S	S	36
				18	60	S	S	S	60
				36	84	S	S	S	94
Dilution 1–6 with acid	650	6.174	0.031	S	S	66	S	S	
				S	S	S	S	S	
				S	S	S	S	S	
Dilution 1–12	350	9.254		68	60	95	84	36	
				72	84	S	84	95	
				S	108	S	96	36	
Serum control				S	36	84	96		
				S	S	96	76		
				S	S	96	96		
				1–10,000,000			1–1,000,000		
Control organism				24			24		
				36			36		

Numbers refer to hours of survival.

* 0.2 cc. injected of undiluted sample.

others. The serum of this experiment protected against a million lethal doses in a dilution of 1–160, whereas the second fraction protected in a dilution of 1–1280, thus showing per cc. a concentration of eight times. However, this concentration is only relative, inasmuch

as the volumes of the different fractions as compared with the original serum are not the same. It is to be noted that in the second fraction less protein, as judged by nitrogen content, was found in the protective dose than in the other fractions.

The different fractions with Type II serum were obtained in a different way: namely, (1) by dilution 1–6 with distilled water; (2) by adding 120 cc. of N hydrochloric acid to 16 liters of the supernatant from the 1–6 dilution; and (3) by diluting this supernatant with an equal volume of water making a final dilution of 1–12. As seen in Table III, the greatest amount of antagonistic substance was shown to be in the first fraction, a dilution of 1–6; while in the second there was no demonstrable amount of this substance; in the third it is difficult to say whether there was any protection or any antagonistic substance. Comparison of the protective value of the serum with the different fractions shows here as with the Type I a relative concentration of eight times. The precipitate of the third fraction, the one lacking protection, is comparable to the euglobulin precipitated from normal serum by dilution in water with addition of acid.

With Type III the fractions were obtained in the same manner as with Type I except that, owing to the absence of precipitate with a 1–10 dilution, the third fraction is made by adding 30 cc. of N hydrochloric acid to 15 liters of this 1–10 dilution. The results with this type, seen in Table IV, are somewhat different from those with Types I and II, in that the antagonistic substance seems to be present in the acid fraction. But as, in the other types, the maximal amount of protective substance is found in the second fraction. Here again there is a relative concentration of eight times of the protective substance.

It is to be emphasized that these are single examples and they do not necessarily illustrate a general rule. For some samples of sera react to give the maximum of protective precipitate by simple dilution with water and others by dilution with water plus acid, the amount of acid varying with the sera from different horses or from horses at different stages of immunization. In general it may be stated that, in the case of potent Type I serum, the best results are obtained, as judged by the minimum nitrogen content in a protective dose, by a dilution with water of 1–10 to 1–20. However, it has been observed

that, with weak Type I serum or with serum from horses having an excessive amount of antigen, a dilution of 1–1 or less, with water, yields a precipitate having an antagonistic effect, whereas subsequent dilutions of 1–10 or more, with or without acid, yield a protective precipitate practically free from this action. When acid is used, the final product, as also seen from Chart I, contains a high content of

TABLE IV

Various fractions of water-insoluble precipitate (Type III)

(All mice received injections of 0.5 cc. of an 8-hour broth culture diluted 1–100)

FRACTION	VOLUME	MILLIGRAMS NITROGEN PER CUBIC CENTIMETER	NITROGEN IN PROTECTIVE DOSE	DILUTIONS OF SAMPLES							
				0.2*	1–10	1–20	1–40	1–80	1–160	1–320	1–640
Dilution 1–1	44	10.66	0.066	S	66	96	S	S			
				S	S	S	S	S			
Dilution 1–5	76	9.74	0.03	S	S	S	S	S	S	48	28
				96	S	S	S	S	S	60	36
Dilution 1–10 with acid	32	18.11	0.056	66	84	96	S	S	S		
				84	96	S	S	S	S		
Serum control				S	S	S	84				
				S	S	S	96				
	1–10,000,000			1–1,000,000							
Control organism	24			26							
	36			36							

Numbers refer to hours of survival.

* 0.2 cc. injected of undiluted sample.

nitrogen per unit of protection, due, we believe, to an admixture with inert euglobulin. It may be stated that with the sera from seven Type III horses complete precipitation of the protective substance has been obtained by dilution with water of 1–6 with the addition of 10 cc. of N hydrochloric acid to a liter of serum. Less satisfactory results have been secured from Type II than from the other types

of sera. However, with potent sera of this type a yield as high as 70 per cent of the protective substance has been obtained.

EXPERIMENT IV. TOXIC SUBSTANCE IN ANTIPNEUMOCOCCUS SERUM

In our work on the antibody of antipneumococcus sera we have readily confirmed the observations of Chickering (5) in which he showed the lack of parallelism between dose of serum and mouse

TABLE V

Toxic substance in Type I immune serum

(All mice received injections of 0.5 cc. of an 8-hour broth culture diluted 1–10)

SAMPLE	DILUTIONS OF SAMPLES				
	0.5*	1–10	1–20	1–40	1–80
Precipitate from 1–1 dilution	18	48	S	S	36
	18	52	S	S	60
Portion soluble in 1 per cent NaCl (Berkefeld)	36	S	84	18	36
	38	S	S	84	36
	0.5 cc.		0.25 cc.		
Toxicity of suspended precipitate	18		18		
	18		S		
Toxicity of NaCl portion	18		S		
	18		S		
	1–10,000,000		1–1,000,000		
Control organism	36		36		
	36		36		

Numbers refer to hours of survival.

* 0.5 cc. injected of undiluted sample.

survival with a constant number of organisms. For example, a large dose of serum (0.5 to 0.75 cc.), depending upon the sample, may fail to protect against one or even ten million MLD. Whereas 0.2 cc. of the same serum would protect against one to ten million MLD. Furthermore, in antibody solution prepared by the dilution method, especially in the case of Types II and III, this phenomenon appeared to be much more marked. Indeed it seemed logical to suspect the presence in such precipitates of a toxic substance as well as the protec-

tive antibody. The fact that it has been possible to prepare antibody solution comparatively free from this toxic substance may possibly preclude this toxicity being an inherent characteristic of the protective antibody. Yet it should be borne in mind that other immunological manifestations may perhaps be due to the presence of this antagonistic or toxic substance.

This experiment is given merely to show, in immune serum precipitated by dilution with water, the presence of a substance which is lethal for mice. Examples from Types I, II, and III sera are given. The sample of Type I serum was from a group of horses which had received excessive doses of antigen; and although of sufficient potency to pass the usual standard, it failed to yield a highly protective antibody solution. The precipitate was obtained by dilution of 20 liters of serum with an equal volume of distilled water. After being in the ice-box overnight the supernatant was siphoned off and the precipitate collected by rapid centrifugalization and suspended in 100 cc. of 1 per cent sodium chloride. This suspension was shaken for two hours and divided into two portions: one was centrifugalized and the supernatant run through a Berkefeld candle; the other was saved as a suspension for tests.

As can be seen from Table V, two types of tests were made; namely, estimation of protective antibody against one million MLD, and toxicity. The results are quite clear. Both in the case of the suspension and in the sample passed through a Berkefeld candle, 0.5 cc. of the undiluted sample proved lethal, and in a dilution of 1–10 exhibited sufficient toxic action to neutralize the protective antibody.

In case of the Type III serum the same general procedure was carried out with 5 liters of serum, except that the suspended precipitate only was tested. Undoubtedly, as seen from Table VII, there is present in this fraction of serum a substance which antagonizes the animal defense and is also capable of producing death without the aid of the infecting organisms.

The precipitate from Type II serum was obtained by the same method as that for Type I and III, 8 liters of serum being used. In the first place (Table VI) it is to be noted that the same toxic substance was found as in the case of the other immune sera. From the consideration that the precipitate from the 1–1 dilution is sparingly soluble

in neutral salts and is toxic in itself for mice, a possible connection is seen between this and the precipitate obtained by means of the soluble specific substance of pneumococci. For as given in another publication (9), the latter precipitate was found to be sparingly soluble in neutral salts, and in large doses displayed action antagonistic to defense. To make a further comparison of these, the dissociability of

TABLE VI

Toxic substance in type II immune serum

(All mice received injections of 0.5 cc. of an 8-hour broth culture diluted 1–100)

SAMPLE	DILUTIONS OF SAMPLES						
	0.5*	1–5	1–10	1–20	1–40	1–80	1–160
Precipitate from 1–1 dilution	18	20	36	132	S	S	S
	18	48	60	132	S	S	S
Precipitate soluble in M/100 Na_2CO_3 54°C. for 30′	36	S	S	S	S	20	24
	84	S	S	S	S	26	36
Same unheated	20	36	24	24	36	36	24
	36	36	24	24	36	36	36
Portion of precipitate insoluble in Na_2CO_3	18	18	108	S	S	S	S
	18	18	S	S	S	S	S

	1 cc.	0.5 cc.	0.25 cc.
Toxicity† 1–1 precipitate	18	18	S
	18	18	S

	1–10,000,000	1–1,000,000
Organism control	36	36
	36	36

Numbers refer to hours of survival.

* 0.5 cc. injected of undiluted sample.

† Without organism.

the precipitate from the 1–1 dilution of immune serum was tested. For as shown by the work of Chickering (5), Huntoon (6), who used respectively dissolved pneumococci and living pneumococci as antigens, and also by our results (9) with the soluble specific substance, the precipitate obtained by any one of these three precipitating agents may be efficaciously dissociated by heat in the

presence of M/100 sodium carbonate. Hence, an attempt was made to effect dissociation of the precipitate from the 1–1 dilution in the following way: a portion of the original precipitate was shaken two hours in M/100 sodium carbonate, centrifugalized, and one-half of the supernatant heated for 30 minutes at 54°C., the other half being used for a control. The precipitate of the unheated sample was also tested. As can be seen from Table VI, the supernatant of the unheated sample failed to show any protective value, whereas the residuum insoluble

TABLE VII

Toxic substance in Type III serum

(All mice received injections of 0.5 cc. of an 8-hour broth culture diluted 1–100)

FRACTION	DILUTIONS OF SAMPLES				
	0.5*	1–10	1–20	1–40	1–80
Precipitate from 1–1 dilution	18 18	20 36	S S	S S	36 48
	0.5 cc.		0.25 cc.		0.125 cc.
Toxicity†	84 18		18 36		70 36
	1–10,000,000			1–1,000,000	
Control organism	20 24			24 36	

Numbers refer to hours of survival.

* 0.5 cc. injected of undiluted sample.

† Without organism.

in carbonate had less toxic or antagonistic action than the original precipitate. On the other hand, the heated supernatant exhibited definite protective value, but still contained some substance antagonistic to the animal defense. The same results and the same problem are given with the precipitate obtained by the soluble specific substance. It may be thus assumed that the two precipitates are similar, prnot identical. For both are relatively insoluble in neutral salts, itoduce action antagonistic to the defense of the animal, and are her dissociated or detoxified by using carbonate with heat.

EXPERIMENT V. EFFECT OF REPRECIPITATION FROM SALT SOLUTION BY DILUTION IN WATER

In another publication (2) it was observed that the protective substance of Type I was essentially freed from phosphorus by reprecipitation of a sodium chloride solution by dilution in water, a method used by Sorensen (10) in purifying euglobulin; but that the protective property was destroyed markedly by this process. The earlier experiment was carried out at room temperature. It seemed possible that this destruction might be prevented if the same process was carried out at a lower temperature. Accordingly, samples of precipitate of the different types of serum were dissolved in 1.5 per cent

TABLE VIII

Effect of reprecipitation from salt solution by dilution in water

REPRECIPITATION	MILLIGRAMS NITROGEN PER CUBIC CENTIMETER				DILUTIONS GIVING POSITIVE REACTIONS											
					Type I				Type II				Type III			
	I	II	III	Normal	Protection	Agglutination	Precipitin	C.F.*	Protection	Agglutination	Precipitin	C.F.	Protection.	Agglutination	Precipitin	C.F.
First	3.9	2.1	1.8	2.2	320	240	160	10	80	80	40	10	2	40	20	2
Second	3.06	1.4	1.6	2.1	320	200	140	10	160	60	20	5	10	40	15	2
Third	2.8	1.6	1.6	1.8	320	200	140	20	160	60	30	5	10	30	15	2
Fourth	3.0	1.9	1.6	1.8	320	180	140	20	160	60	30	2	2	30	10	2
Fifth	2.8	1.5	1.5	1.05	320	180	140	20	80	40	20	2	2	30	10	2

* C.F. indicates complement fixation.

sodium chloride and this solution diluted with eight volumes of water. The resultant precipitate, collected by centrifugalization, was dissolved in 1.5 per cent sodium chloride solution and, as in the first reprecipitation, this solution diluted with eight volumes of water. The process was continued five times. Samples from each reprecipitation were saved for the usual nitrogen, protection, agglutination, precipitin, complement-fixation determinations. The precipitin test was carried out by the soluble specific substance isolated from Types I, II, and III, according to the technique of Heidelberger and Avery (12). In another study, in collaboration with Dr. Bailey (11), the question of the different immunological manifestations of the water-

insoluble precipitate from antipneumococcus serum is taken up in detail. However, in that study the effect on the common immunological reactions of reprecipitation from sodium chloride solution, as in this experiment, was not estimated. Hence these tests were applied at the various stages of reprecipitation as indicators of antibody destruction. The results, as can be seen from Table VIII, show that all the tests gave a positive reaction varying principally in the titer; complement-fixation lowest, precipitin next, agglutination next, and finally, protection the highest. A general parallelism, was found in all the tests with perhaps the exception of protection in the case of Type II. In this case there was an increase in the protective value, extending throughout the second, third and fourth precipitation, with a low value in the original material and in the fifth or final sample. There was also some decrease in nitrogen as well as agglutination and precipitin titer in this sample but not in the same proportion as in protective value. The possibility is thus seen of reprecipitation from a sodium chloride solution of the protective protein by dilution with water with but slight loss in potency so long as the process is carried out at a low temperature (8°C. in this experiment). This experiment gives additional evidence, though by no means conclusive, in support of the unitarian conception of antibodies, sponsored by Bordet (13), Nicolle (14), Dean (15), Zinsser (16), and others, that a single antibody gives rise to the common immunological manifestations.

EXPERIMENT VI. SOLUBILITY OF THE WATER-INSOLUBLE PRECIPITATE AT VARIOUS CONCENTRATIONS OF HYDROGEN ION

This experiment deals with the solubility of the final precipitate of Experiment V at various hydrogen-ion concentrations in a sodium hydroxide-hydrochloric acid mixture. The procedure was as follows: To a measured portion of precipitate suspended in water from each type of sera, I, II, and III as well as normal euglobulin which had undergone the same treatment, sufficient N/20 hydrochloric acid was added to make a clear solution. Then from a test sample from each of the four solutions the amount of N/20 sodium hydroxide which would dissolve the acid solution of protein was estimated. Now, the minimum solubility point lies between the hydrogen-ion concentration of the acid solution and that of the alkaline solution. To obtain

this point, because of the slow rate of solution equilibrium of these protein solutions, it becomes necessary to make several estimations. The technique found practical was as follows: first a division of the acid solution into the desired number of parts, and then addition to each successively of an increment of alkali. The increment was estimated by dividing the alkali required to dissolve one of the samples by the number of members of the series, in this case, ten. For clarity, the details of the Type I are given only as an example: 220 cc. of the precipitate was dissolved in 22 cc. of N/20 hydrochloric acid. This solution was centrifugalized at high speed and the clear supernatant was divided into eleven portions. The amount of alkali necessary to dissolve one portion was 8 cc. of N/40 sodium hydroxide. This volume divided by ten, the number in the series, is the increment, namely 0.8 cc. It is thus seen that we have equal portions of protein at different hydrogen-ion concentrations from the acid to the alkaline solution. After the addition of alkali, the volumes of the various tubes were made the same with water and the series shaken in a mechanical shaker for four hours. The precipitates were finally collected by rapid centrifugalization for one hour; the supernatants being measured and saved for tests, and the precipitates dissolved in sufficient 1.5 per cent sodium chloride to make the original volume. Precipitin tests and agglutination reactions were done only on the dissolved precipitate.

A glance at Chart II, which depicts the results on Type I precipitate, shows that nitrogen content, protection, and agglutination titer run, in general, a parallel course in the middle portion of the curve. However, at both extremes there is a pronounced disproportion in this respect; on the alkaline side the protective value is low per milligram of nitrogen, while on the acid side it is relatively high. These results are in keeping with other observations to the effect that dilute acid is less destructive to the protective antibody than is alkali. There is a zone of minimum solubility extending from 3.2 to 4.8 cc. of alkali. However, the supernatant from the sample to which 4.0 cc. of alkali were added contains the least amount of nitrogen. Thus, the supernatants from the samples 3.2, 4.0, and 4.8 cc. of alkali are respectively 0.048, 0.039, and 0.179 mgm. of nitrogen per cubic centimeter. Although it has been difficult to determine exactly the hydrogen-ion

concentration of a solution containing such a small amount of protein, by the use of dilute indicators the pH of the sample containing the least amount of protein was judged to be between 6.6 and 6.8.

The experiment with Type II precipitate, although in the main the same, shows certain differences from that with Type I. The differences are for the most part concerned with the degree of solubility. For as seen from Chart III, the zone of minimal solubility extends from the 3.2 cc. sample to the 4.8 cc. one, with but little difference in nitrogen content. It is also apparent, as judged from the curve of solubility at the various hydrogen-ion concentrations that this precipitate is more soluble on the acid than on the alkaline side. However, the least amount of nitrogen per cubic centimeter is found in the sample neutralized with 4.0 cc. of alkali. The hydrogen-ion concentration of this sample is approximately pH 6.6. In the case of protective value, as in Type I precipitate, the acid side gives the larger value per milligram of nitrogen, indicating either a destruction of the antibody or a precipitation by alkali. The dissolved precipitates were not tested.

The sample of precipitate from Type III serum used in this experiment contained antibodies of such low value that tests were made on dilutions of 1–1, 1–2, 1–5, etc. up to 1–10. However, reactions were definite and are given in Chart IV. Here it is seen that protective value and nitrogen content run an almost ideal course both on the acid and alkali sides, in this respect differing from the results obtained from Types I and II precipitates. For on the alkaline side in these instances disproportion was shown between nitrogen content and protective value. In case of Type III precipitate the minimal solubility lies between the sample of 1.6 and 2.4 cc. of alkali. The hydrogen-ion concentration, as with Types I and II, approximates pH 6.6.

The results of the experiment with euglobulin, run in parallel with the immune serum, will be given in a more complete study. However, it may be stated that at the hydrogen-ion concentration of minimal solubility the euglobulin contained in the supernatant 0.14 mgm. of nitrogen per cubic centimeter, whereas the corresponding supernatants from immune sera contained only: Type I, 0.04, Type II, 0.01, and Type III, 0.03 mgm. per cubic centimeter, showing a much lower solubility than the sample of euglobulin.

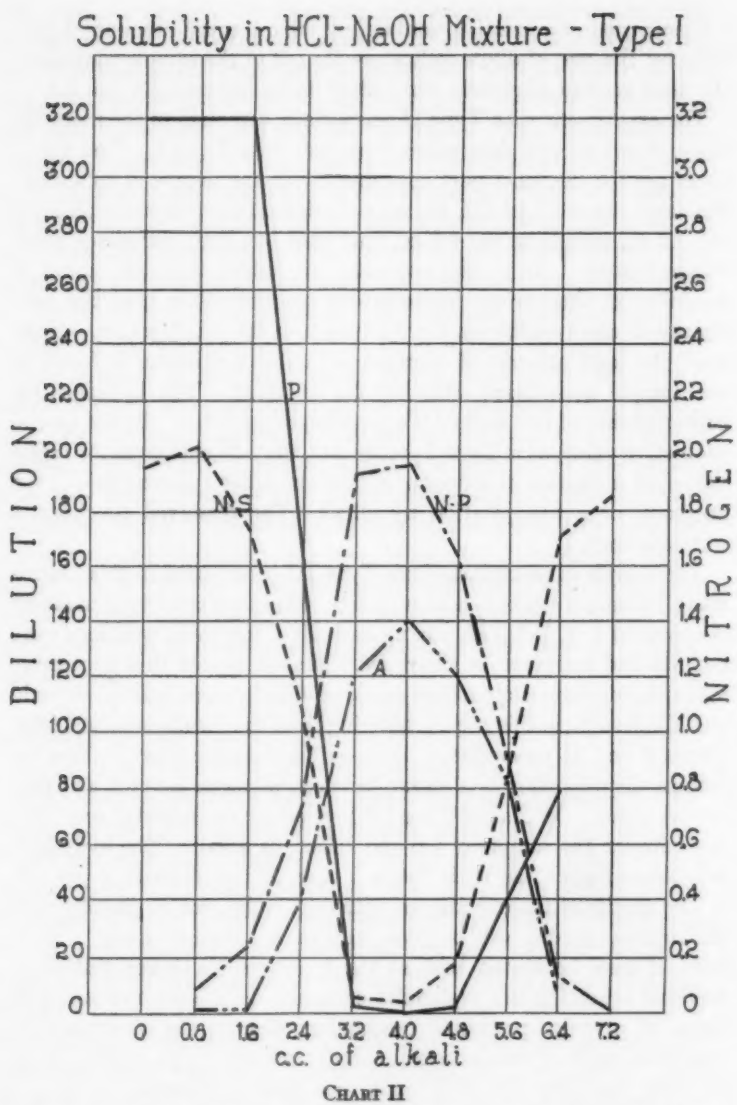

CHART II

A, agglutination of dissolved precipitate; *P*, protection of supernatant; *NS*, milligrams of nitrogen per cubic centimeter of supernatant; *NP*, milligrams of nitrogen per cubic centimeter of dissolved precipitate.

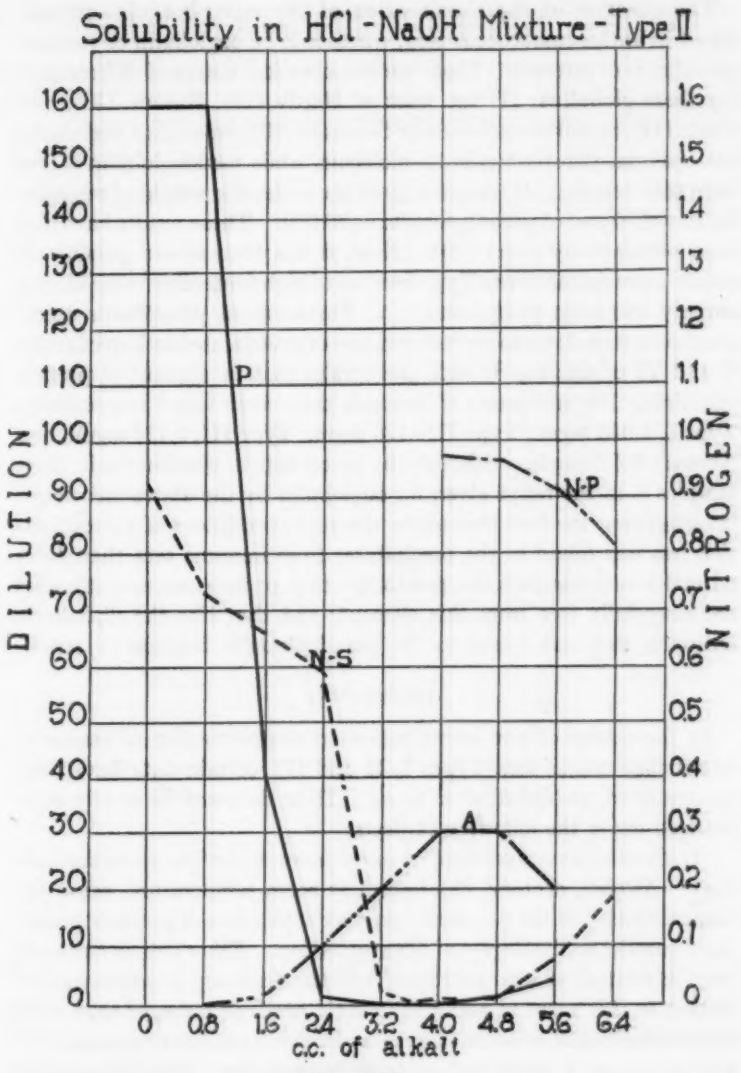

CHART III

A, agglutinaion of dissolved precipitate; *P*, protection of supernatant; *NS*, milligrams of nitrogen per cubic centimeter of supernatant; *NP*, milligrams of nitrogen per cubic centimeter of dissolved precipitate.

The question of the classification of the water-insoluble protein found in antipneumococcus sera, whether it be euglobulin or pseudoglobulin, is of interest. There are two chemical means of differentiating these globulins: (1) the work of Hardy (17), Haslam (18), and Chick (19), recently confirmed by Sorensen (10), shows that euglobulin has essential phosphorus in its molecule, while pseudoglobulin is free from this element; (2) Sorensen gives the molecular weight of euglobulin as 40,000 and of pseudoglobulin as 80,000. These results have also been obtained by Cohn (20). Now, it has been shown possible to obtain a precipitate from Type I serum of high protective value almost entirely free from phosphorus (2). Furthermore, phosphorus determinations were done on the four samples of protein used in Experiments V and VI of this report with the results that in amount of protein containing 100 milligrams of nitrogen there were found respectively: Type I, 0.048 mgm., Type II, 0.126 mgm., Type III, 0.125 mgm., and Normal, 0.257 mgm. Although the percentage of phosphorus in these samples is in the range given for euglobulin by the above-mentioned investigators, the fact that under the same conditions of reprecipitation less was found in the precipitates from immune sera than from normal serum suggests the possibility that pneumococcus antibodies are essentially free from this element; and that like the diphtheria antitoxin they are found in the pseudoglobulin fraction of serum.

DISCUSSION

In the course of our investigation on the protective substance in antipneumococcus sera Types I, II and III, certain data have been accumulated, published or in press. These data can be briefly summarized under the following subjects:

(1) *Conditions or agents which cause destruction of the protective antibody.* Alcohol, acetone, chloroform at room temperature, cause the denaturization of the protective protein with a loss of potency practically parallel to the degree of denaturization. Ether acts in the same way in neutral or acid solutions, but the antibody is somewhat resistant to this agent in alkali (2). Acid or alkali stronger than N/20 hydrochloric acid or N/20 sodium hydroxide at room temperature or ice-box causes a more or less rapid destruction. The substance is thermolabile. Pepsin, trypsin, and pancreatin entirely destroy the

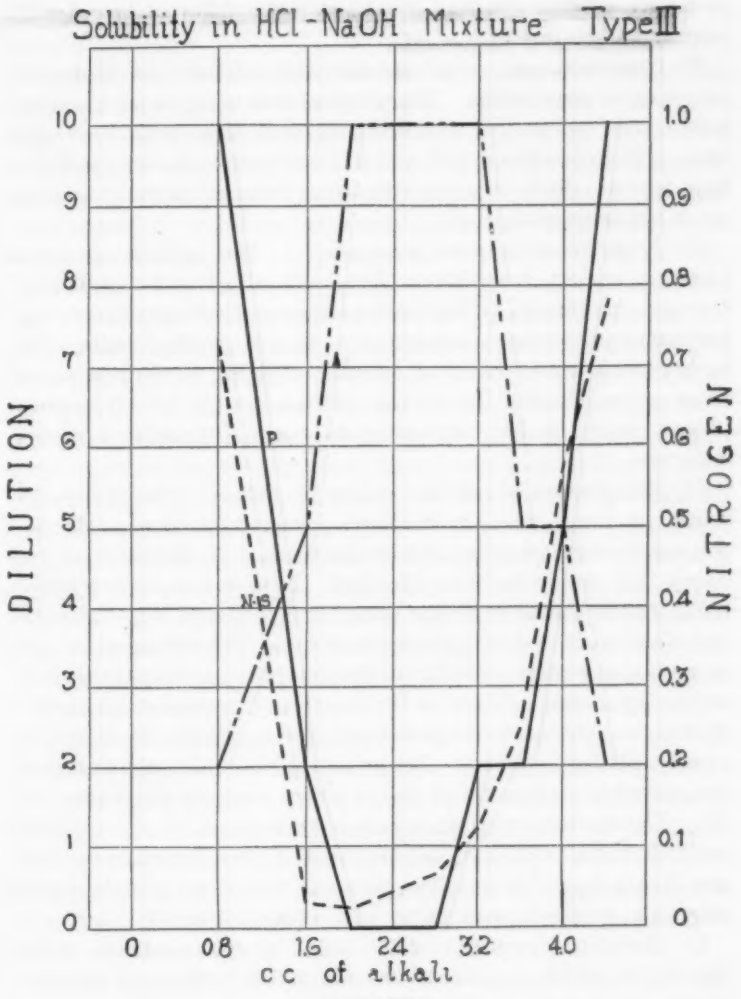

CHART IV

A, agglutination of dissolved precipitate; *P*, protection of supernatant; *NS*, milligrams of nitrogen per cubic centimeter of supernatant.

protective substance, pepsin the most rapid, followed in order by trypsin and pancreatin.

(2) *Conditions under which the protective substance can be handled with minimal deterioration.* Reprecipitation as many as ten times has been carried out at 8°C. with acid and alkali at no greater strength than N/20 hydrochloric acid and N/20 sodium hydroxide; and also from neutral salts by dilution with water, when the process is carried on at low temperatures (2).

(3) *Properties of the protective substance.* The antibody, as far as known at present, is soluble in dilute acid, alkali, and neutral salts. It is also precipitated by ammonium sulfate and sodium sulfate at approximately the same concentration as that of pseudoglobulin. The hydrogen-ion concentration of minimal solubility in the presence of some sodium chloride lies between pH 6.7 and pH 6.8. The phosphorus content is low, suggesting no essential phosphorus in the molecule.

(4) *Precipitation of antibody by specific antigen.* Precipitates obtained by living, dead, or dissolved pneumococci, also by the specific soluble substance prepared by the method of Heidelberger and Avery (12), are similar if not identical. In each case the precipitate is but slightly soluble in neutral salts, but readily soluble in dilute acid and alkali when neutral salt content is low. The hydrogen-ion concentration of minimal solubility of the precipitate in hydrochloric acid-sodium hydroxide mixture, is the same as, or approximates closely, that of the water-insoluble protein obtained by diluting immune sera, namely, pH 6.6 to pH 6.8. The soluble specific substance in correct concentration precipitates all the protective antibody in immune sera (9). The resultant precipitate may be dissociated by any of several methods, sodium carbonate and heat being the best so far encountered, and the product of dissociation, with but little of the soluble specific substance, precipitated by 18 per cent sodium sulfate (9).

(5) *Biological significance of the soluble specific substance.* Injection of the soluble specific substance along with relatively avirulent pneumococci results in the death of mice with a smaller number of organisms than the control. This paralysis of the animal defense is apparently specific to type. The soluble specific substance of pneumococcus, both *in vitro* and *in vivo*, neutralizes the protective antibody.

The product of neutralization is a compound sparingly soluble in neutral salts (9).

To make significant deductions from the results thus briefly summarized would at this time be impossible. It is of importance, however, that a substance isolated from the pneumococci, essentially a carbohydrate, neutralizes, both *in vitro* and *in vivo*, the substance in immune serum which protects the animal against multiple lethal doses of pneumococci. Furthermore, it is of interest that this soluble specific substance, or it may be a toxic material in some way combined with it, weakens the defense of the animal. A consideration of these facts with the results of the important work of Zinsser and Tamiya (21) and of Avery and Heidelberger (22), reported contemporaneously, that the soluble specific substance is in itself devoid of immunizing power but nevertheless is an essential constituent of bacterial products, makes it apparent that in the blood stream of actively immunized animals the protective substance is at least partially neutralized by a soluble specific substance. If the neutralization is complete, a compound is formed which is lacking in protective value. However, when this compound is dissociated, either within or without the animal, the antibody is free to protect. That dissociation takes place *in vivo* has been found experimentally by us and may be inferred from the observations of Dochez and Avery (23) and of others, in which they reported the presence of the soluble specific substance in the urine of pneumonia patients. The view of Cole (24) in this connection is significant. For he noted the neutralization of protective antibody by infected exudates and sera containing large amounts of "soluble substance" and suggested that his results offer an explanation for the fact that infection of partially immunized animals tends to remain localized while definite septicemia results only after the soluble substances have accumulated in the blood stream in sufficient amounts to neutralize the specific antibodies. It is thus seen that the results of our experiments support the views of Cole (24).

Results referred to in another paper and repeated later show that perhaps the antibody itself, when not combined with some portion of antigen or soluble specific substance, may be soluble in water, as well as in neutral salts. If it is assumed that the combination between antibody and soluble specific substance is a chemical one and also that

the uncombined antibody is soluble in water, it does not seem unreasonable that the soluble specific substance, when attached to only one or more of the many possible free chemical groups of antibody, produces the change in its solubility to the degree that it is no longer soluble in water. If the combination is more complete, there is still further alteration in solubility and the fully neutralized compound is not only insoluble in water, but also in neutral salts. If this thought is pursued still further and applied to precipitins and agglutination reactions, it becomes quite obvious that, when sufficient antigenic substance is added to serum, it combines with the antibody and gives rise to the compound insoluble in neutral salts. In that sense, if one takes into consideration the unexplained prozone effect in agglutination and precipitin reactions, then the reactions properly carried out give a measure of the antibody content of the serum.

In studying the preparation of the protective substance given in this paper and in others, it is not inferred that we are dealing with an essentially pure solution of the antibody. This is believed especially from experiments of precipitation with soluble specific substance in which all the protective substance was neutralized and precipitated and yet there remained in the supernatant as much as 20 to 40 per cent of the original protein as judged by the nitrogen content. This contamination is in our opinion the classical euglobulin in the greater part with an admixture of some pseudoglobulin.

SUMMARY

(1) The amount of precipitate obtained from single samples of Types I, II and III antipneumococcus serum by dilution with water is much greater than from normal serum. Furthermore, the yield of precipitate at various hydrogen-ion concentrations shows that the zone of maximal precipitation of the immune is to the alkaline side of that of normal serum. There exists a broad zone of hydrogen-ion concentration at which, on dilution, the protective antibody of immune serum is precipitated; thus Type I, pH 7.8 (dilution in distilled water) to pH 5.5; Type II, pH 7.8 to pH 5.5; and Type III, pH 7.8 to pH 6.2. Protection did not run a parallel course with the amount of precipitate.

(2) The optimal yield of precipitated substance obtained by dilution

with water in the samples of serum, Types I, II and III used in Experiment II, was found to be: Type I, 1–18 or 1–20; Type II, 1–14 to 1–18; and Type III, 1–12 to 1–14. There was a partial correlation only between protein content and protective value. The result with potent Type I serum is thought to be a general rule, whereas an insufficient number of different Type II and III sera have been tested to warrant a definite conclusion in this respect.

(3) Dilution of the three types of sera 1–1 with water yields a precipitate having relatively low protective value and in the case of Type I and II sera, shown in Experiment III, contains a substance antagonistic to the animal defense. Type III precipitate under these conditions showed no demonstrable antagonistic action. On further dilution of the serum (1–5 to 1–12) more precipitate was obtained which contained less toxic substance than the first fraction, and gave the highest protective value, while the precipitate obtained by adding acid to the supernatant sera from the previous dilutions by water contained low protective value and in the case of Type III serum a toxic substance.

(4) A substance which was lethal for mice was found in Types I, II and III sera. In the case of Type II serum, precipitate heated for thirty minutes at 54°C. in the presence of M/100 sodium carbonate results in either a detoxification or a dissociation, thus liberating the bound protective antibody.

(5) Samples of precipitate from Type I, II and III sera were dissolved in sodium chloride and reprecipitated by dilution in water five times at 8°C. The final precipitate contained agglutinin, precipitin, complement-fixing bodies and protective action per milligram of nitrogen only to a slight degree less than the original.

(6) The samples of precipitate of the different types of sera and normal serum reprecipitated five times (Experiment V) gave a minimal solubility of: Type I, 0.04, Type II, 0.01, Type III, 0.03, and normal serum 0.14 mgm. of nitrogen per cubic centimeter, showing that this sample of euglobulin was four to ten times more soluble than the precipitates from antipneumococcus sera. The hydrogen-ion concentration of minimal solubility of the precipitates from immune serum in the presence of some sodium chloride lies between pH 6.6. and pH 6.8.

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STUDIES ON SUPRA-VITALLY STAINED LIVING AND DEAD LEUCOCYTES AND EPITHELIAL CELLS IN THE VAGINA OF THE RAT AND THEIR RELATION TO THE OVULATION CYCLE[1]

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For many years all workers on problems of reproduction have realized the importance of finding some external evidence through which to date a recurrent phase of the ovarian cycle. If such an event as the rupture of the ripe follicle could be diagnosed accurately without the necessity of sacrificing the animal, we should have a definite starting-point for many biological experiments. In 1917, Stockard and Papanicolaou (1) introduced the technique of the vaginal smear. By the microscopic examination of the vaginal cell content in the guinea-pig, these observers were able to foretell accurately the period of oestrus, which is followed within a few hours by ovulation. In 1919 and 1920 Long and Evans (2) used this technique in a brilliant study of the oestrous cycle of the rat. In both of these rodents the oestrous vaginal smear is characterized by the presence of non-nucleated epithelial cells desquamated from the wall of the vaginal canal. This phase of the cycle can be differentiated with certainty from that of prooestrus and dioestrus, for in the former the smear is characterized by the presence of many nucleated epithelial cells, and in the latter the presence of the leucocyte is diagnostic.

The studies of all observers on vaginal cell cycles in the various mammals have been made on stained dead cells. At Dr. F. R. Sabin's suggestion I undertook an analysis of the living vaginal cells. We were interested in knowing whether the leucocytes of the vaginal canal are living, and if so, whether they have a cycle of activity. We also wished to know whether living epithelial cells could be differentiated from dead ones, and whether clasmatocytes or tissue wandering cells

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occur in the vaginal secretion. Only through a knowledge of such questions can we begin to understand the physiology of the cell cycle in the vagina. In addition, there are certain mammals, the most important of which are primates, which do not have so obvious a vaginal cell cycle as the guinea-pig, rat and mouse. It is possible that certain facts learned from the study of living vaginal cells will be of use in determining the time of ovulation in the monkey and other primates.

The procedure of supra-vital staining has come into very general use through the publications of Arnold, Maximow, Sabin and others. The technique consists in exposing a thin film of living cells outside of the body to a moderately concentrated solution of one of the vital dyes. Some of the dyes stain the intra-cellular granules and vacuoles of the living cells, while other dyes stain the mitochondria. The supra-vital dyes most commonly used are neutral red for the staining of cell granules and vacuoles, and janus green for a staining of the mitochondria. By observing the differences in staining of different kinds of cells, combined with a study of the appearance of the cytoplasm and nucleus, living cells can be differentiated. In addition, from the study of the supra-vital stains, the finer cellular histology can be observed and the variations in the cell pictures can be correlated with the physiology of the cell.

For my observations sexually mature albino rats were used. The preparations are made by pipetting into the vagina, and immediately withdrawing, about 1 cc. of a 1 to 10,000 solution of neutral red made up in normal saline. Through this method a sample of vaginal cells in even suspension is obtained; and then a drop of this cell suspension is pipetted on a very clean cover-slip. The cover-slip with its drop of fluid is inverted and placed with care upon a slide, previously prepared according to Sabin's (3) directions for supra-vital blood films, with a 1 to 5000 alcoholic neutral red. Because of the toxicity of janus green and because it was found unnecessary for these studies, I usually omitted this dye. When I used janus green, one drop of a super-saturated alcoholic solution was added to 2 cc. of a 1 to 5000 alcoholic neutral red, and with this the slides were then flooded. The American dyes were used in this work and found to be entirely satisfactory; though a greater dilution is necessary than for the German

dyes. In sealing the preparations I merely rim the edge of the cover-slip with oil-immersion cedar oil, for it was found to be more satisfactory than vaseline, because if the objective runs over the edge of the cover-slip in the cedar-oil preparation, the lens is not clouded on contact with the oil, as with vaseline. The cedar oil is also more easily applied and the preparations can be rimmed without any danger of injuring the cells by making pressure on the cover-glass. I have kept preparations sealed with cedar oil for over 8 hours without any evidence of toxicity to the cells or any drying up of the film. The supra-vitally stained smears are studied in a warm box kept constantly at 37.5°C. A differential count is made on 200 cells studied with an apochromatic oil-immersion lens and a high power compensating ocular. In some preparations, especially those obtained from the dioestrus, part of the film is made up of solid masses of cells immeshed in mucus. These fields are avoided in making counts, separate cells only being tabulated. Even, thin films of the vaginal cells are obtained with great ease and regularity, and with much less difficulty than blood films.

Long and Evans (4) in their monograph on the oestrous cycle in the rat have shown that the dehiscence of the vaginal epithelium takes place at about the same rate over the entire mucosal surface, a fact which accounts for the homogeneous cell picture which the vaginal smear presents. The authors in their discussion first consider the dioestrous interval, or the period during which the female reproductive tract is in a state of relative quiescence, this phase occupying about one-half of the rat's 4 or 5 day oestrous cycle. The vaginal smear during this period contains leucocytes and small irregularly shaped epithelial cells, immeshed in a thin, somewhat stringy mucous secretion. The epithelial cells during the stage, which these authors designate as Stage 5, occur singly, never in groups. Stage 5 is followed by Stage 1, or the period of prooestrus. A smear taken at this time shows a complete absence of leucocytes, but great numbers of small round, nucleated epithelial cells of strikingly uniform appearance and size are present. The oestrous phase, Stage 2, or the period during which the female is willing to mate, is characterized by cells markedly different from those of Stage 1; for now the smear contains large, thin, transparent, scale-like elements—the cornified cells. Stage 3, or the

postoestrus, is distinguished by a cell picture which is merely an exaggeration of Stage 2, for we continue to find only cornified cells, but in greatly increased numbers—the cells occurring in sheets and singly. Stage 4, the transition phase between the postoestrus and dioestrus, is inaugurated by the appearance of leucocytes among the cornified cells and ends with the disappearance of the latter.

In our observations on the living vaginal cells we have separated the cells into 3 groups: epithelial cells, white blood cells and tissue wandering cells.

EPITHELIAL CELLS

The epithelial cells are of two main types, the cornified or non-nucleated epithelial cell and the nucleated epithelial cell. The cornified cell is a large flat, transparent cell, with sharp angular edges. I have never seen this cell stain with neutral red. The second type of epithelial cell is a thick-edged, granular, round, nucleated cell which may or may not be vacuolated. Some of the cells of this group stain with neutral red while others do not; but by the appearance of the unstained cells one cannot foretell which of the nucleated epithelial cells will stain and which will not stain with the dye. In those epithelial cells which stain with the dye. there is a staining of fine dots, vacuoles and rod-shaped small bodies in the cytoplasm. Some of these cells stain yellow or alkaline with the indicator-dye, neutral red, while others take a red or acid stain. There is never any staining of the nucleus in the epithelial cells. Nucleated, granular, epithelial cells occur in abundance immediately before oestrus, and they reappear after oestrus. However, it is usually only the early dioestrous, nucleated epithelial cell which stains with neutral red. This probably indicates that only a very fresh, living, epithelial cell stains with the supra-vital dye. When the cornified layer sloughs following oestrus, there remain beneath it several layers of nucleated epithelial cells; however, it seems, that the upper layers of these epithelial cells, the postoestrous epithelial cells, do not stain with the dye. These cells are probably injured or killed by the same process which cornified the more superficial cells. Later, in the early dioestrous phase of the cycle, after the cornified cell layer, and the more superficial, apparently injured, nucleated layers

have sloughed away, some of the fresh nucleated epithelial cells are shed into the vaginal canal, and in our preparations some of them stain with neutral red. Apparently, after these granular nucleated cells have been exposed to the vaginal secretion for a short time, they refuse to take up the dye, which we think, in this class of cells, indicates cell death or injury.

WHITE BLOOD CELLS

The white blood cells of the vagina are largely polymorphonuclear leucocytes. At a certain stage of the oestrous cycle these leucocytes are living and actively motile; however, the usual motility seen in these saline-neutral red films is somewhat different from that shown by the leucocytes of supra-vital blood films. In the saline-neutral-red preparations most of the leucocytes remain stationary, and merely extrude and draw in their pseudopodia with great speed, there being as many as 15 separate pseudopodia to a cell. Yet in these films of vaginal cells some of the leucocytes show the progressive motility characteristic of the leucocyte in blood films; progressive motion, with the nucleus posterior, the width of the cell less than its length, and a streaming of the granules and cytoplasm. Other leucocytes stained with neutral red in saline are round, and the granules, instead of streaming, merely showed confused Brownian motion. In order to determine if this atypical motility was specific for some of the vaginal leucocytes or merely the result of our technique, we examined vaginal cells without the addition of normal salt solution, and also blood cells in normal saline. The vaginal leucocytes in their own secretion moved like blood cells in serum films; while, on the other hand, the blood cells in salt solution behaved like the suspension of vaginal leucocytes stained with neutral red made up in saline. The addition of the saline to the vaginal secretion is necessary, however, in order to obtain a thin even film of the cells; the vaginal secretion alone being too thick and sticky.

There seems to be an activity cycle of the vaginal leucocytes which has apparently a direct relation to the oestrous cycle. Immediately after oestrus, almost all of the leucocytes are of the actively motile variety. Toward the middle of the rat's 4 or 5 day ovulation cycle, cells appear in large numbers which seem to be dead leucocytes. They are naked, granular, semi-opaque looking, polymorphic nuclei entirely

unstained with neutral red. Various transitions between the healthy living leucocyte and the dead leucocyte occur. As the cycle progresses the relative number of dead leucocytes increases, while the number of live leucocytes decreases. Actively motile leucocytes occur whose cytoplasm and nucleus stain deeply with neutral red; these we interpret, following the work of Winternitz and Evans (5), as injured cells, possibly injured through our technique. We have the impression that these leucocytes with stained nuclei occur in relatively greater numbers toward the close of the dioestrous interval when the dead leucocytes are particularly abundant, and it therefore appears that the leucocyte is more susceptible to injury at this time. One often finds a field of a dozen healthy leucocytes with one injured polymorphonuclear in their midst, a fact which points to a graded susceptibility to injury among the leucocytes themselves.

Without attempting to uphold either the unity or duality of the origin of the cell granules and vacuoles, I have distinguished the granules from the vacuoles in the vaginal leucocytes. It appears that the amount of vacuolization of the leucocytes in the vagina also varies with the phase of the ovulation cycle. Immediately after oestrus there are few or no stained vacuoles in the cytoplasm of the leucocytes; while very heavily vacuolated polymorphonuclears are the rule at the end of the dioestrous period. The fine, faint granules of the polymorphonuclear leucocyte of the rat stain similarly in all periods of the cycle. Both the granules and the vacuoles of the vaginal leucocytes stain a scarlet or an orange in supra-vital preparations with the indicator-dye neutral red; rarely, if ever, a true yellow.

On the basis of the observations of a rather constant increase in both the relative number of dead leucocytes and the amount of cell vacuolization as the oestrous cycle progresses, it seems that the leucocytes probably migrate into the vaginal canal in one large group at stage 4 of the cycle and the beginning of stage 5; additional ones continually invading, but in much smaller numbers. If the leucocytes migrated into the vagina at a perfectly uniform rate at all periods of the cycle, except oestrus, we should expect at all times a constant proportion of dead to live, and vacuolated to non-vacuolated leucocytes. In place of this we find a constant relative increase of both dead and vacuolated polymorphonuclears from postoestrus to pre-

oestrus. It seems to me that there is a sudden invasion of the canal by leucocytes; these cells soon beginning their phagocytosis of the bacteria, cells and debris. Apparently, after a day or two these leucocytes are injured and die, their cytoplasm dissolving and leaving the naked nuclei. On the other hand the rats, whose ovaries have been removed and which have no oestrous cycle, show a fairly constant relative proportion of live and dead, vacuolated and non-vacuolated leucocytes; in them the invasion of the vagina by the leucocytes must be a continuous, even process.

In addition to the polymorphonuclear leucocyte, the vaginal secretion of the mature rat also contains eosinophiles, lymphocytes and monocytes. These three types of cells never occur in large numbers, never more than 4 per cent of the total cell count; nor does their presence bear any discoverable relation to the oestrous cycle.

In the vaginal smear of the female from which the ovaries have been removed, motile and dead leucocytes are always present in varying proportions. The living leucocytes are, however, usually more abundant than the dead ones. In these oöphorectomized rats, cornified cells are rarely present in the vaginal secretion, and if present, only one or two cells in a microscopic field. Since in these animals from which the ovaries have been removed there is no consistent or marked cornification of the epithelial cells, and since living leucocytes are constantly present in the vaginal canal in as great numbers as in the normal unoperated female, we may deduce that the leucocyte does not cause the cornification of the epithelial wall; and, furthermore, that the cornified cells are not the only stimulus which will cause leucocytes to migrate into the vagina.

CLASMATOCYTES OR TISSUE WANDERING CELLS

Since it is well known that clasmatocytes, or tissue wandering cells, occur in the peritoneal fluid, we were interested to determine whether or not they occurred in the vaginal fluid. In order to demonstrate beyond question their presence in the vaginal fluid, we injected two rats intraperitoneally with a 1 per cent aqueous solution of trypan blue. At the first injection the animals were given 1.5 cc. and then two injections of 1 cc. on three successive days. Two days after the first injection the vaginal fluid of one of the animals contained un-

mistakable clasmatocytes, while the second rat showed clasmatocytes on the fourth day following the first injection. Clasmatocytes are constantly present during the dioestrous phase of the cycle, comprising 0.5 to 2 per cent of the vaginal cells; however, the clasmatocytes are absent during the oestrous phase. In their morphology the clasmatocytes of the vagina are similar to those described for the connective tissues of the rat by Evans and Scott (6). Many of the clasmatocytes are very large and all have vacuoles of various sizes and staining reactions.

THE DIFFERENTIAL COUNTS OF VAGINAL CELLS

In differential counts of the supra-vitally stained vaginal films there is a great variation in the percentage of the several types of cells present, these variations in the percentage of the different cellular elements having a definite correlation with the oestrous cycle. In our differential counts we tabulated five groups of cells separately: the living white blood cells and tissue cells (a group composed largely of polymorphonuclear leucocytes, but also containing occasional lymphocytes, monocytes, clasmatocytes and eosinophiles), dead white blood cells, cornified epithelial cells, nucleated epithelial cells stained, and nucleated epithelial cells unstained with neutral red. Three sexually mature females were studied for several weeks, a daily differential of 200 cells being counted for each.

I shall describe a typical oestrous cycle of Rat 107, beginning with stage 3 of Long and Evans, or that phase in which the smear contains almost only cornified vaginal cells. On this day there were 95 per cent cornified cells, 4 per cent nucleated epithelial cells unstained, and 1 per cent of nucleated stained epithelial cells; leucocytes both dead and living were absent. On the following day the cornified cells were fewer, having decreased to about 20 per cent, while the unstained nucleated epithelial cells had increased to about 70 per cent. White blood cells appeared at this time in a proportion of 8 per cent of the total, 4 per cent living and 4 per cent dead. This stage, at which the vaginal secretion contained cornified and nucleated epithelial cells in large number with a few leucocytes, corresponds to stage 4 of Long and Evans. Twenty-four hours later, with the animal in full dioestrus, or stage 5 of Long and Evans, I found over 50 per cent of motile

living white blood cells, and no dead white cells. Cornified cells had decreased until they made up but 5 per cent of the total cell count. The unstained nucleated epithelial cells had also decreased to 15 per cent. At this mid-point of the cycle most of the nucleated epithelial cells stained with neutral red which in the cycle described reached 25 per cent of the cells counted. On the second day of the dioestrus the living leucocytes were still more abundant than all the other cells added together. The nucleated and cornified cells were

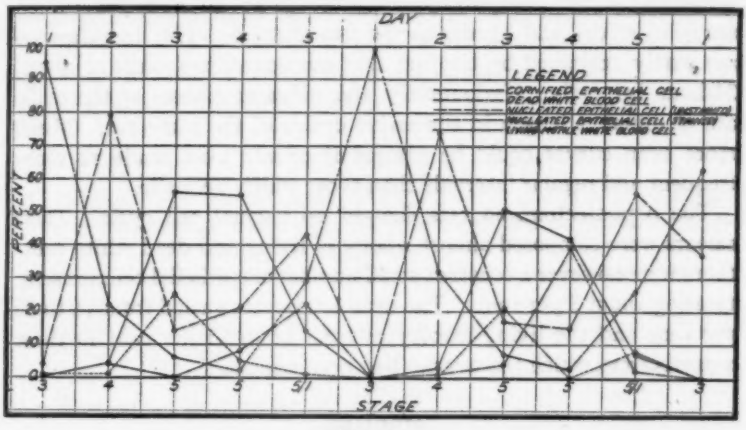

FIG. 1

about the same in number as on the previous day when the stained epithelial cells had markedly increased. On this day the dead white cells began to increase, having become 8 per cent of the total cell count. Twenty-four hours later, or on day 5, the dead white cells reached their maximum of 22 per cent and the living leucocytes decreased to 14 per cent. Both cornified and nucleated epithelial cells materially increased. This count is typical of the transition phase between stages 5 and 1 of Long and Evans.

These supra-vital differential counts have shown the existence of an interesting relation of the leucocytes and epithelial cells to the phase of ovulation. During oestrus the leucocytes are absent from the vagina, to return in late postoestrus and reaching their maximum in early dioestrus. During postoestrus and early dioestrus the

leucocytes are all living, motile, sparsely vacuolated cells. In the middle of dioestrus dead leucocytes appear, increasing as the cycle progresses. The dead leucocytes are most abundant at the end of dioestrus. The living leucocytes of middle and late dioestrus are heavily vacuolated. The epithelial cells are usually unstained with neutral red except during early dioestrus when some of the nucleated epithelial cells stain quite brilliantly with the supra-vital dye.

In the chart we give two successive cycles of Rat 107 in exact detail. These may be taken as quite typical, although not all the cycles studied are so clear cut and regular as the two charted. The irregularities are partly explained by the fact that our counts are made at about 24-hour intervals. Undoubtedly the different phases of the cycle are of different length in successive cycles of the same rat. Much more even curves could be plotted if counts were made at more frequent and regular intervals than those which we made.

The oöphorectomized rats showed no vaginal cell cycle. Their smears were composed almost wholly of living and dead white cells; there were however a constant small number of epithelial cells present, totaling about 5 per cent. The dead leucocytes varied from 14 to 56 per cent and the living leucocytes from 42 to 85 per cent in these animals from which the ovaries had been removed.

SUMMARY

We have described a simple and satisfactory method of studying the living cells of the vaginal fluid. By means of this technique we have determined that the leucocytes of the vaginal fluid are living and motile. In addition to the living leucocytes there are also dead ones, the relative proportion of dead to living leucocytes increasing as the cycle progresses from postoestrus through late dioestrus. The amount of vacuolization of the polymorphonuclear cells of the vagina also bears a definite relation to each phase of the ovulation cycle. In addition to the polymorphonuclear leucocytes we find a small number of eosinophiles, lymphocytes and monocytes. The nucleated vaginal epithelial cells take the supra-vital stain only at a certain period of the oestrous cycle, these cells at other periods remaining unstained in our preparations. By the vital staining of rats with

trypan blue we were able to demonstrate the presence of clasmatocytes among the vaginal cells.

It is hoped that the method and facts observed will be useful in determining the time of ovulation in those animals which have not so obvious a vaginal cell cycle. In the monkey, for instance, as shown by Corner (7), there is no such sharp variation in the number of leucocytes and epithelial cells as in the rat and guinea-pig, which allows us to determine ovulation, but perhaps in this and similar primate species a study of the vaginal smear by such a technique as we have applied to the rat may bring out the existence of a cyclic difference in the state of the leucocytes or epithelial cells which is not discernible by the study of dead cells.

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STUDIES OF CERTAIN HITHERTO UNDESCRIBED ANTIRACHITIC SUBSTANCES

ANDOR DE BOSANYI

In two papers published during the last year some experimental data were presented concerning the production by normal bone marrow of an organic factor, which promotes the deposition of lime salts in cartilage and bone. In these communications it was shown that watery extracts of defatted marrow caused healing when they were administered to rickety animals.

Since the marrow from which these extracts were made had been defatted and the extracts themselves were subjected to dialysis, the organic matter in them must have consisted in a large part of protein. With this in mind it seemed logical to assume that the factor in the extracts which induced healing of rickets was a protein or a substance closely related to protein, and this assumption formed the starting point of the experiments which are reported below.

Bone marrow consists for the most part of fats, proteins and inorganic salts. Among the proteins haemoglobin, histons, nucleoproteids, fibrinogen and globulins are the most notable. It contains also some xanthin bases, cholesterin, inosite and lactic and uric acids. Brown and Guthrie further claim to have found certain pressor and depressor substances in bone marrow, the action of which was entirely similar to that of adrenalin.

In order to determine to which of these substances the specific antirachitic effects were due, rats which had been made rachitic by feeding them with McCollum's rickets-producing diet No. 3143 were treated with these different materials given separately. Treatment was first attempted with haemoglobin. When the gelatine in the rickets-producing diet was replaced with this substance, no rickets developed in rats so fed and growth was practically the same as in animals maintained on McCollum's stock food, and they were able to bear and rear young.

The lowest proportions of haemoglobin necessary to prevent rickets

was from 5 to 6 per cent by weight of the diet. This amount of haemoglobin will, moreover, cure rickets; or in other words will cause the deposition of calcium in the bones when fed to rachitic animals. Smaller amounts of haemoglobin will not completely prevent rickets from developing, although 2 per cent will permit some deposition of calcium in the metaphysis if fed to a rachitic animal.

Van Leersum has stated recently that haematoporphyrin will cure rickets when injected into rachitic rats. He believed that the curative effect was the result of sensitization of the animals to light—a well known action of haematoporphyrin. Van Leersum's hypothesis made it necessary to ascertain whether or not haemoglobin acted in the same way. With this end in view a group of rachitic rats were treated with haemoglobin in a light-tight compartment. They were treated for eight days with 5 per cent haemoglobin. At the end of this time they were examined and showed quite as marked healing as was found in a control group treated with haemoglobin in room light.

The haemin group of the haemoglobin, in the form of haematin, is not effective as an antirachitic. Fifty milligrams of this substance injected subcutaneously failed to cause healing of rickets in rats, and the same substance given in the drinking water to the amount of 5 per cent was quite without avail in so far as the cure of the rickets was concerned. These experiments show that the antirachitic effect of the haemoglobin is not only independent of the illumination to which the animals treated with the substance are exposed, but also that the chromophor group is inactive as an antirachitic substance.

The globin fraction of haemoglobin is commonly regarded as a histon. Histons differ from other proteins chiefly in regard to their content of diamino acids. The histidin content is especially large, amounting to 12 per cent. Groups of animals treated with histidin, arginin, and lysin are now being studied, but it is not possible at this time to report the results of the treatment of rickets with these substances. Five per cent by weight of histamin in the diet, however, causes healing of rickets in 6 to 8 days. Cystin may also be an effective antirachitic.

Since the presence of pressor and depressor substances in the bone marrow has been reported, the administration of adrenalin was undertaken. Rachitic rats were treated with this substance as prepared for

commerce, and with a crystalline product, some of which was kindly given me by Prof. J. J. Abel. The drug was given first in high dilution (1–20,000) and the strength was gradually increased until the animals were taking it in a concentration of 1–5,000. These solutions were given the animals as their drinking water. After 4 to 5 days of this treatment, the rickets of the treated animals began to heal and the rats developed a tolerance for adrenalin. They were able to stand the subcutaneous injection of 0.5 to 1.0 mgm. adrenalin, a dose which would have been lethal without previous desensitization. After two subcutaneous injections, or after drinking a 1–2000 solution of adrenalin for 2 days, healing of their rickets began. It is of interest to oote that the mono-amino acid tyrosine which has a structure very similar to that of adrenalin has no action on rickets even though given in amounts as great as 4 to 5 per cent of the total food intake.

Since histamine, which has shown positive antirachitic effects, is an imidazol derivative, a group of rickety animals was treated with an alkaloid having the imidazol group in its composition and a biological action similar to that of adrenalin, viz., pilocarpin. This alkaloid was alsδ found to be antirachitic.

It is interesting to note that in rats with beginning healing rickets, as the result of treatment with adrenalin or pilocarpin, there was no increase in the inorganic phosphorus of the blood serum, when compared with that in the rachitic control animals. Control rats had for example an inorganic serum phosphorus of 2.35 to 3.2 mgm. per 100 cc. of serum. Two rats treated with adrenalin had 2.0 and 2.97 mgm. and a rat treated with pilocarpin, 2.9.

Rickety rats treated with a diet containing 5 per cent chlorophyl showed healing of their rickets, but these animals, although they did not starve, lost a great deal of weight and died in about two weeks as the result of some toxic action of the unpurified chlorophyl, so that no conclusions can be drawn from this experiment.

In conclusion it may be said that highly acidified or alkalinized food had no effect in controlling rickets in my experimental animals.

CONCLUSIONS

1. Healing of rickets may be induced by certain hitherto unrecognized antirachitic substances. These are:

2. A histon—the globin of haemoglobin, a diamino acid—cystin, an aromatis compound—adrenalin, and an alkaloid, pilocarpin.

THE JOHNS HOPKINS MEDICAL RESEARCH CLUB

Abstracts of Papers Presented

On the Mechanism of the Jaundice Following Pulmonary Infarction in Patients with Heart Failure. By Arnold Rice Rich and William H. Resnik. *From the Departments of Pathology and Medicine, Johns Hopkins University. Presented May 15, 1925.*

It is well known that patients suffering from cardiac decompensation have, almost invariably, a plasma jaundice and, not infrequently, a scleral jaundice. It is also recognized that in such patients a general tissue jaundice may appear shortly after pulmonary infarction. The jaundice developing in this latter circumstance has been thought to be the result of absorption of bilirubin formed from haemoglobin locally in the infarct. However, microscopical studies of such infarcts show clearly that very little change takes place in the extravasated erythrocytes for a number of days following the infarction, and they do not at all support the idea that local formation and subsequent absorption of bilirubin proceeds at a rate sufficiently rapid to be responsible for the early jaundice occurring in these cases. On the other hand, it is a perfectly familiar fact that chronic passive congestion severely injures and often destroys many liver cells about the efferent veins of each lobule, and this injury, we believe, lies at the bottom of the plasma jaundice accompanying cardiac decompensation, in that the excretory power of the liver is decreased and the organ becomes unable to eliminate completely the increased amount of bilirubin known to be formed in patients with failing heart. The cause of the liver damage in chronic passive congestion has been a matter of discussion for some time, the principal theories being (1) that the cells are damaged by pressure from the dammed-back blood, and (2) that they are damaged by the deficient supply of oxygen.

In an attempt to find a pathological basis for the inability of the liver to excrete the increased amount of bilirubin formed in certain anaemic states, Rich and Bumstead have observed that in pernicious as well as in secondary anaemias the cells about the efferent veins of each liver lobule may be damaged in a manner often indistinguishable from that accompanying chronic passive congestion, and the lesion has been produced experimentally in animals by simple haemorrhage. This lent support to the conception that the damage to the liver in chronic passive congestion may be referable more to poor oxygenation than to pressure; and it seemed probable, therefore, that the intensification of the plasma jaundice to the point of tissue jaundice following pulmonary infarction in cardiacs might be merely the result of an intensification of the oxygen deficiency effect of chronic passive congestion, leading to a further lowering of the excretory efficiency of the

liver and a consequent retention of bilirubin in the blood stream to the point of staining the tissues. That pulmonary infarction does intensify the oxygen deficiency effect of chronic passive congestion might be expected, from the fact that areas of lung tissue are suddenly thrown out of function, and that, when infarction is extensive, an added burden is suddenly thrown upon the right heart in the effort to force the blood through the restricted, already congested, capillary bed of the lungs. And indeed, Binger, Brow and Branch have demonstrated experimentally that anoxemia is a prominent effect of pulmonary infarction.

It was our purpose in the present experiments to test first the hypothesis that mere absorption of bilirubin formed locally in moderate-sized haemorrhages could bring about jaundice in patients with chronic passive congestion. To this end we injected into the thigh muscles of patients suffering from cardiac decompensation 50 to 100 cc. of blood removed from their own veins. This procedure reproduces, in a tissue with a rich vascular supply and noted absorbing power, the local conditions of a moderately large pulmonary infarction *without introducing the factors of changes in respiration and circulation.* The bilirubin content of the circulating blood was estimated by the van den Bergh method at frequent intervals over a period of days following the injection of blood. In eight such experiments we found no instance in which there was any evidence that absorption of bilirubin or blood pigment from the intramuscular haemorrhage increased the bilirubin content of the patient's blood. Consequently, we do not regard the factor of absorption from the haemorrhage as the cause of the intensificaton of the blood bilirubin retention following pulmonary infarction in cardiacs.

We cannot yet state definitely the mechanism which underlies the jaundice in these cases. Further experiments must be carried out; but such evidence as we have points strongly to the probability that the jaundice is referable to an accentuation of functional and anatomical damage to the liver resulting from the increase in anoxemia attendant upon pulmonary infarction.

BOOK REVIEWS

The Surgery of Pulmonary Tuberculosis. By JOHN ALEXANDER. 356 pp. $4.50. (*Lea and Febiger, Philadelphia and New York, 1925.*

This is the first publication in the English language to present the entire subject of the development and present status of a recent but broadening field of surgery. There are many physicians and surgeons in this country who are still unaware that surgery is now curing or improving approximately two-thirds of those selected cases of far advanced tuberculosis that other methods of treatment have failed to benefit.

This is a book which should be read by every medical student, by every physician and by every surgeon, who is qualified by training to do thoracic surgery.

S. J. C.

Osler's Modern Medicine. Third Edition, re-edited by Thomas McCrae, M.D., assisted by Elmer H. Funk, M.D. Volume I. 845 pp. $9.00. *Lea and Febiger, Philadelphia, 1925.*

Among the many systems of medicine which have appeared in recent years "Osler's Modern Medicine" has assumed a place of distinct value, and one, therefore, approaches the first volume of the new edition with great interest.

As McCrae points out in his preface, no radical alterations have been made in the plan of the work and the changes consist essentially in revising and bringing up to date the articles of the older editions.

One is pleased to see that the plan of treatment aims to give a more or less comprehensive review of the natural history of the various diseases rather than merely to sketch the present status or recent developments in a subject, as has been done in articles in certain other systems of medicine. The main purpose of this sort of production must always be to furnish a store-house of information to which one can go to get concrete facts and figures.

Naturally, there is some variation in the merit of the several articles and one is impressed, for example, by the fact that only a very brief sketch is devoted to so important a disease as poliomyelitis, in contrast to the highly satisfactory exposition of typhoid fever. The article on toxemia, septicemia, and pyemia, which follows the traditional treatment of this subject, makes one wish for an adequate description of the septic infections which, as far as the reviewer knows has never been done in English. The monograph by Lenhartz in Nothnagel's System and the briefer articles based on it by Schottmüller in Mohr and Staehlein's System and by Jochmann in his textbook illustrate what can be done in this domain, although something a little more in accord with recent discoveries is needed.

Finally, the reviewer wonders why the articles occur in apparently haphazard sequence, with bacillary dysentery preceded by gonococcus infections and followed by tularemia, with scarlet fever towards the end of the volume and erysipelas some dozen articles ahead of it. This is not an important point, but if the gonococcus and meningococcus infections are grouped, why not place the hemolytic streptococcus infections—erysipelas, scarlet fever, and tonsillitis—likewise in some approximation?

On the whole, the work is one of undoubted merit and will continue to hold its place in the foremost ranks of *Systems* of this sort.

A. L. B.

Gynecologic Urology. By LYNN LYLE FULKERSON. 247 pp., with 166 illustrations. $6.00. (*Philadelphia, P. Blakiston's Son & Co., 1925.*)

The author's statement that one-fifth of all gynecological patients present urinary symptoms is certainly not an exaggeration. Hence there would seem to be a definite field for a work dealing with the interrelationships of gynecology and urology, and the present volume would be more valuable if more stress had been laid upon the manner in which the two specialties overlap. To mention but one, perhaps the most common, instance, many an unnecessary abdominal operation is done when the real lesion is a ureteral calculus, a ureteral stricture, or a chronic pyelitis. And yet such problems receive little emphasis in the book, which is almost "straight urology."

The other of the two chief faults lies in the sketchiness of treatment of even the most important topics, making the book little more than a synopsis of the subject. While most authors are apt to be too verbose, Fulkerson goes to the opposite extreme. We question whether one will gain any really worthwhile impression from the author's description of "elusive ulcer," or of the Kelly method of ureteral catheterization, or of many other topics touched upon. On the subject of mercurochrome and hexylresorcinol he is not an optimist. Concerning the former he rather caustically comments: "Recognized medical schools should submit drugs synthetized in their laboratories to unbiased clinical test in other institutions before permitting their exploitation by commercial houses." This book has its value as a methodic outline of the subject, but that is about all.

E. N.

Parasitology for Medical Students. By ALEX MILLS KENNEDY. First Edition. 142 pages. $3.00. (*Oxford University Press: Humphrey Milford, London, 1925.*)

This little book is very well written. It takes up about all the parasites ordinarily found in practice; but they are rather briefly covered and not enough attention is given to diagnostic procedures. Concentration methods of ova and cysts are only considered in the case of hookworm and there the brine flotation test, a very important one, is omitted. The illustrations are for the most

part excellent. It can be recommended as a compact and handy reference work rather than as a textbook.

H. G. M.

An Intermediate Textbook of Physiological Chemistry. By C. J. V. PETTIBONE. 404 pp. $3.25. (*C. V. Mosby Co., St. Louis, 1925.*)

This is a useful book, packed full of information. It includes a general survey of the field of physiological chemistry and a laboratory manual. It has much to recommend it. It is concise, up to date, well organized, and well written. Certain of the discussions, for instance, that of the polariscope, are excellent from the pedagogical point of view. The author states that he has "attempted to avoid confusing the beginners with lengthy discussions of debated points, but to set forth as clearly as possible the present status of our knowledge." In this he has succeeded, but in so doing he has written a book which is inadequate as a text for many classes of students, for instance, for medical students. This is true for several reasons. It does not satisfy an enquiring mind and does not stimulate thought. For the most part it states information, with too little attempt to show how this knowledge was obtained. Discussions of certain important compounds, such as the purines and pentoses, are conspicuously inadequate.

M. V. B.

An Introduction to Objective Psychopathology. By G. V. HAMILTON, M.D., Director of Psychobiological Research, Bureau of Social Hygiene, Inc., New York City, N. Y. 354 pp. $5.00. (*St. Louis, The C. V. Mosby Co., 1925.*)

The book represents the intimate experience of a year's neuropsychiatric missionary work in an Ohio town of 30,000 inhabitants, by a physician with long research experience in animal psychology. He gives a presentation of 200 cases, not in terms of disease-entities but as persistent nonadjustive affective reactions to physical discomforts and disabilities, baffled major cravings, impairments of advantage, economic difficulties, fear-inciting stimulations; indirect reactions to sexual urges, masturbation, inferiority, conditioned reactions, submissive reactions to inferiority, and others. He adds neurological cases such as could be found in the rank and file of patients in any neuropsychiatric practice.

After some 200 pages of records the author offers a fragmentary discussion of "objective psychopathology" with particular, not especially correlated and unified, chapters on the theroretical neurological and neurophysiological foundation, "endocrinology," behaviorism, comparative studies of reactions to baffling disadvantages, habit-formation, "relation of inhibition of responsiveness to indirect responsiveness," unsatisfied major cravings, reactions to inferiority, and sexual behavior.

The book is offered to physicians, social workers, and lay readers with a view to focussing attention upon the importance of method in psychopathologic re-

search. A foreword by his friend, Yerkes, urges that "every physician, every teacher, everyone who deals with problems of personnel should read the book." As a rather inadequately systematized "Spoon River alive," with cases in the main sensibly and objectively handled, with very personal and often anecdotal experiences from the laboratory of a monkey colony, from reminiscences of his own and those of visitors to the laboratory, and observations of the countryside and the physician's office, the book can hardly be said to do justice to the scientific ambitions of the author or the needs of those to whom it is recommended; unless we assume that there is a call for somewhat indiscriminate stimulation of a promiscuous group of readers. In a limited circle of workers in the field there might possibly be a realization of what Yerkes says in the preface:

"Though little known and less understood by psychologists and psychopathologists, Hamilton's discoveries are undoubtedly of fundamental and far-reaching importance. I am old enough to be rash on occasion, so I venture to predict that he will presently find himself the leader of a school of psychopathology which will as importantly modify our current conceptions of reaction tendencies and their relations, as has psychoanalytic method our notions about mental content. My friend is too modest and distrustful of his insights to believe what I am writing, but those of his colleagues who are blessed with brains which guarantee open-mindedness presently will." And

"The world is so full of ordinary books which picture the perfection of mediocrity and of conventional-mindedness—termed by our author most appropriately 'sheepishness'—that it is a great pleasure by contrast to present to readers who have learned to expect little originality, constructiveness, or novelty in point of view, a book which applies the principles of objective and comparative psychology to psychopathology."

A. M.

BOOKS RECEIVED

Oxford Medical Publications. Publisher: Humphrey Milford, London. The following fourteen volumes:

The Chemical and Physiological Properties of the Internal Secretions. By E. C. Dodds, Ph.D., B.Sc., M.B., B.S., and F. Dickens, M.A., Ph.D. 1925. 8°. 214 pages. $2.50.

Midwifery Mechanics. By Andrew Buchanan, I.M.S. (Retd.), M.A., M.D., M.Ch., M.A.O. 1924. 8°. 82 pages. $2.50.

Clinical Researches in Acute Abdominal Disease. By Zachary Cope, B.A., M.D., M.S. (Lond.), F.R.C.S. (Eng.). 1925. 8°. 148 pages. $4.00.

Artificial Sunlight and Its Therapeutic Uses. By Francis Howard Humphries, M.D. (Brux.), F.R.C.P. (Edin.), M.R.C.S. (Eng.), L.R.C.P. (Lond.), L.M. (Rot., Dublin), D.M.R. & E. (Cantab.). Illustrated. 1924. 8°. 203 pages. $2.75.

Parasitology for Medical Students. By Alex. Mills Kennedy, M.D. (Glas.). 1925. 8°. 142 pages. $3.00.

The Nursing of Eye Cases. By LOUISE KINGHAM, S.R.N. N.d. 12°. 16 pages. $0.30.

History of Medicine. By DR. MAX NEUBURGER. Translated by Ernest Playfair, M.B., M.R.C.P. In two volumes, Vol. II, Part 1. 1925. 8°. 135 pages. $2.25.

Reports of St. Andrews Institute for Clinical Research, St. Andrews, Fife. Vol. 2. 1924. 8°. 190 pages. $3.00.

Radium. Its Therapeutic Uses in General Practice. By G. H. VARLEY, M.D. (Oxon). 1924. 8°. 103 pages. $1.75.

Middle Age and Old Age. By LEONARD WILLIAMS, M.D. 1925. 8°. 296 pages. $3.25.

The Early Diagnosis of the Acute Abdomen. By ZACHARY COPE, B.A., M.D., M.S. (Lond.), F.R.C.S. (Eng.). Third edition. 1925. 8°. 233 pages. $3.80.

The Histology of the More Important Human Endocrine Organs at Various Ages. By EUGENIA R. A. COOPER, M.D. 1925. 8°. 119 pages. $4.00.

Neurological Fragments. By J. HUGHLINGS JACKSON, M.D., F.R.S., F.R.C.P. With Biographical Memoir by James Taylor, M.D., F.R.C.P. And including the "Recollections" of the late Sir Jonathan Hutchinson and the late Dr. Charles Mercier. 1925. 8°. 227 pages. $3.75.

Diseases of the Heart. By SIR JAMES MACKENZIE, F.R.S., M.D., F.R.C.P., LL.D. (Ab. & Ed.), F.R.C.P.I. (Hon.). Fourth edition. 1925. 8°. 496 pages. $9.00.

AN OSLER MEMORIAL VOLUME

The International Association of Medical Museums will shortly issue a memorial volume of appreciations, anecdotes and reminiscences of Sir William Osler by over a hundred colleagues, friends and pupils. The volume is prefaced by forewords from Professor William H. Welch and by the late Sir Clifford Allbutt, followed by some 600 pages of memorial contributions, with over 60 engravings and half-tone illustrations. At the end, there is a complete classified bibliography of the writings of Osler, based upon the chronological bibliographies already published by Miss Minnie W. Blogg (Baltimore) and Dr. Maude E. Abbott (Montreal), followed by a bibliography of "Writings about Osler," and a list of advance subscribers. The Osler bibliography is as complete as the labor of several efficient collaborators could make it, and has been extensively annotated, in accordance with Osler's expressed preference for "a combination of biography with bibliography," viz.: "To be of value to the full-fed student of today, a bibliography should be a catalogue raisonné, with judicious remarks and explanations" (1918). The anecdotes, reminiscences and biographical notations in the memorial volume will thus supplement, in a manner, the Cushing biography, which has attracted such widespread interest among the laity as well

as among the medical profession. The present edition of 1500 copies is supported by private advance subscriptions and by a publication fund, inaugurated in January, 1921, by initial contributions from the National Research Council (Washington), the late Sir Edmund Osler (Toronto) and others. Advance subscriptions ($10) may be made by cheque, made payable to the International Association of Medical Museums (Osler Memorial Volume) and addressed to Dr. Maude E. Abbott, Permanent Secretary, International Association of Museums, The Medical Museum, McGill University, Montreal, Canada.